

**INTERLEUKIN-3 (IL-3) MULTIPLE MUTATION POLYPEPTIDES**

This is a continuation-in-part of United States Application Serial No. 07/981,044 filed November 24, 1992 which is incorporated herein by reference.

Field of the Invention

The present invention relates to mutants or variants of human interleukin-3 (hIL-3) which contain multiple amino acid substitutions and which may have portions of the native hIL-3 molecule deleted. These hIL-3 multiple mutation polypeptides retain one or more activities of native hIL-3 and may also show improved hematopoietic cell-stimulating activity and/or an improved activity profile which may include reduction of undesirable biological activities associated with native hIL-3.

Background of the Invention

Colony stimulating factors (CSFs) which stimulate the differentiation and/or proliferation of bone marrow cells have generated much interest because of their therapeutic potential for restoring depressed levels of hematopoietic stem cell-derived cells. CSFs in both human and murine systems have been identified and distinguished according to their activities. For example, granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF) stimulate the in vitro formation of neutrophilic granulocyte and macrophage colonies, respectively while GM-CSF and interleukin-3 (IL-3) have broader activities and stimulate the formation of both macrophage, neutrophilic and eosinophilic granulocyte colonies. IL-3 also stimulates the formation of mast, megakaryocyte and pure and mixed erythroid colonies.

Because of its ability to stimulate the proliferation of a number of different cell types and to support the growth and proliferation of progenitor cells, IL-3 has potential for therapeutic use in restoring hematopoietic cells to normal amounts in those cases where the number of cells has been reduced due to diseases or to therapeutic

treatments such as radiation and chemotherapy.

Interleukin-3 (IL-3) is a hematopoietic growth factor which has the property of being able to promote the survival, growth and differentiation of hematopoietic cells. Among the biological properties of IL-3 are the ability (a) to support the growth and differentiation of progenitor cells committed to all, or virtually all, blood cell lineages; (b) to interact with early multipotential stem cells; (c) to sustain the growth of pluripotent precursor cells; (d) to stimulate proliferation of chronic myelogenous leukemia (CML) cells; (e) to stimulate proliferation of mast cells, eosinophils and basophils; (f) to stimulate DNA synthesis by human acute myelogenous leukemia (AML) cells; (g) to prime cells for production of leukotrienes and histamines; (h) to induce leukocyte chemotaxis; and (i) to induce cell surface molecules needed for leukocyte adhesion.

Mature human interleukin-3 (hIL-3) consists of 133 amino acids. It has one disulfide bridge and two potential glycosylation sites (Yang, et al., CELL 47:3 (1986)).

Murine IL-3 (mIL-3) was first identified by Ihle, et al., J. IMMUNOL. 126:2184 (1981) as a factor which induced expression of a T cell associated enzyme, 20 - hydroxysteroid dehydrogenase. The factor was purified to homogeneity and shown to regulate the growth and differentiation of numerous subclasses of early hematopoietic and lymphoid progenitor cells.

In 1984, cDNA clones coding for murine IL-3 were isolated (Fung, et al., NATURE 307:233 (1984) and Yokota, et al., PROC. NATL. ACAD. SCI. USA 81:1070 (1984)). The murine DNA sequence coded for a polypeptide of 166 amino acids including a putative signal peptide.

The gibbon IL-3 sequence was obtained using a gibbon cDNA expression library. The gibbon IL-3 sequence was then used as a probe against a human genomic library to obtain a human IL-3 sequence.

Gibbon and human genomic DNA homologues of the murine IL-3 sequence were disclosed by Yang, et al., CELL 47:3 (1986). The human sequence reported by Yang, et al. included a serine residue at position 8 of the mature protein sequence. Following this finding, others reported isolation of Pro<sup>8</sup> hIL-3 cDNAs having proline at position 8 of the protein sequence. Thus it appears that there may be two allelic forms of hIL-3.

Dorssers, et al., GENE 55:115 (1987), found a clone from a human cDNA library which hybridized with mIL-3. This hybridization was the result of the high degree of homology between the 3' noncoding regions of mIL-3 and hIL-3. This cDNA coded for an hIL-3 (Pro<sup>8</sup>) sequence.

U.S. 4,877,729 and U.S. 4,959,454 disclose human IL-3 and gibbon IL-3 cDNAs and the protein sequences for which they code. The hIL-3 disclosed has serine rather than proline at position 8 in the protein sequence.

Clark-Lewis, et al., SCIENCE 231:134 (1986) performed a functional analysis of murine IL-3 analogues synthesized with an automated peptide synthesizer. The authors concluded that the stable tertiary structure of the complete molecule was required for full activity. A study on the role of the disulfide bridges showed that replacement of all four cysteines by alanine gave a molecule with 1/500th the activity as the native molecule. Replacement of two of the four Cys residues by Ala (Cys<sup>79</sup>, Cys<sup>140</sup> -> Ala<sup>79</sup>, Ala<sup>140</sup>) resulted in an increased activity. The authors concluded that in murine IL-3 a single disulfide bridge is required between cysteines 17 and 80 to get biological activity that approximates physiological levels and that this structure probably stabilizes the tertiary structure of the protein to give a conformation that is optimal for function. (Clark-Lewis, et al., PROC. NATL. ACAD. SCI. USA 85:7897 (1988)).

International Patent Application (PCT) WO 88/00598 discloses gibbon- and human-like IL-3. The hIL-3 contains a Ser<sup>8</sup> -> Pro<sup>8</sup> replacement. Suggestions are made to replace Cys by Ser, thereby breaking the disulfide bridge,

and to replace one or more amino acids at the glycosylation sites.

EP-A-0275598 (WO 88/04691) illustrates that Ala<sup>1</sup> can be deleted while retaining biological activity. Some mutant hIL-3 sequences are provided, e.g., two double mutants, Ala<sup>1</sup> -> Asp<sup>1</sup>, Trp<sup>13</sup> -> Arg<sup>13</sup> (pGB/IL-302) and Ala<sup>1</sup> -> Asp<sup>1</sup>, Met<sup>3</sup> -> Thr<sup>3</sup> (pGB/IL-304) and one triple mutant Ala<sup>1</sup> -> Asp<sup>1</sup>, Leu<sup>9</sup> -> Pro<sup>9</sup>, Trp<sup>13</sup> -> Arg<sup>13</sup> (pGB/IL-303).

WO 88/05469 describes how deglycosylation mutants can be obtained and suggests mutants of Arg<sup>54</sup>Arg<sup>55</sup> and Arg<sup>108</sup>Arg<sup>109</sup>Lys<sup>110</sup> might avoid proteolysis upon expression in Saccharomyces cerevisiae by KEX2 protease. No mutated proteins are disclosed. Glycosylation and the KEX2 protease activity are only important, in this context, upon expression in yeast.

WO 88/06161 mentions various mutants which theoretically may be conformationally and antigenically neutral. The only actually performed mutations are Met<sup>2</sup> -> Ile<sup>2</sup> and Ile<sup>131</sup> -> Leu<sup>131</sup>. It is not disclosed whether the contemplated neutralities were obtained for these two mutations.

WO 91/00350 discloses nonglycosylated hIL-3 analog proteins, for example, hIL-3 (Pro<sup>8</sup>Asp<sup>15</sup>Asp<sup>70</sup>), Met<sup>3</sup> rhIL-3 (Pro<sup>8</sup>Asp<sup>15</sup>Asp<sup>70</sup>); Thr<sup>4</sup> rhIL-3 (Pro<sup>8</sup>Asp<sup>15</sup>Asp<sup>70</sup>) and Thr<sup>6</sup> rhIL-3 (Pro<sup>8</sup>Asp<sup>15</sup>Asp<sup>70</sup>). It is said that these protein compositions do not exhibit certain adverse side effects associated with native hIL-3 such as urticaria resulting from infiltration of mast cells and lymphocytes into the dermis. The disclosed analog hIL-3 proteins may have N termini at Met<sup>3</sup>, Thr<sup>4</sup>, or Thr<sup>6</sup>.

WO 91/12874 discloses cysteine added variants (CAVs) of IL-3 which have at least one Cys residue substituted for a naturally occurring amino acid residue.



Summary of the Invention

The present invention relates to recombinant human interleukin-3 (hIL-3) variant or mutant proteins (muteins). These hIL-3 muteins contain amino acid substitutions and  
5 may also have amino acid deletions at either/or both the N- and C- termini. Preferably, these mutant polypeptides of the present invention contain four or more amino acids which differ from the amino acids found at the corresponding positions in the native hIL-3 polypeptide.  
10 The invention also relates to pharmaceutical compositions containing the hIL-3 muteins, DNA coding for the muteins, and methods for using the muteins. Additionally, the present invention relates to recombinant expression vectors comprising nucleotide sequences encoding the hIL-3 muteins,  
15 related microbial expression systems, and processes for making the hIL-3 muteins using the microbial expression systems.

The present invention includes mutants of hIL-3 in which from 1 to 14 amino acids have been deleted from the  
20 N-terminus and/or from 1 to 15 amino acids have been deleted from the C-terminus, and in which multiple amino acid substitutions have been made. Preferred muteins of the present invention are those in which amino acids 1 to 14 have been deleted from the N-terminus, amino acids 126  
25 to 133 have been deleted from the C-terminus, and which also contain from about four to about twenty-six amino acid substitutions in the polypeptide sequence. These hIL-3 multiple mutation polypeptides may have biological activities similar to or better than hIL-3 and, in some  
30 cases, may also have an improved side effect profile, i.e., some muteins may have a better therapeutic index than native hIL-3. The present invention also provides muteins which may function as IL-3 antagonists or as discrete antigenic fragments for the production of antibodies useful  
35 in immunoassay and immunotherapy protocols. In addition to the use of the hIL-3 multiple mutation polypeptides of the present invention in vivo, it is envisioned that in vitro uses would include the ability to stimulate bone marrow and

blood cell activation and growth before infusion into patients.

Antagonists of hIL-3 would be particularly useful in blocking the growth of certain cancer cells like AML, CML and certain types of B lymphoid cancers. Other conditions where antagonists would be useful include those in which certain blood cells are produced at abnormally high numbers or are being activated by endogenous ligands. Antagonists would effectively compete for ligands, presumably naturally occurring hemopoietins including and not limited to IL-3, GM-CSF and IL-5, which might trigger or augment the growth of cancer cells by virtue of their ability to bind to the IL-3 receptor complex while intrinsic activation properties of the ligand are diminished. IL-3, GM-CSF and/or IL-5 also play a role in certain asthmatic responses. An antagonist of the IL-3 receptor may have the utility in this disease by blocking receptor-mediated activation and recruitment of inflammatory cells.

#### 20                    Brief Description of the Drawings

Figure 1 is the human IL-3 gene for *E. coli* expression (pMON5873), encoding the polypeptide sequence of natural (wild type) human IL-3 [SEQ ID NO:128], plus an initiator methionine, as expressed in *E. coli*, with the amino acids numbered from the N-terminus of the natural hIL-3.

Figure 2: ClaI to NsiI Replacement Fragment. Figure 2 shows the nucleotide sequence of the replacement fragment used between the ClaI and NsiI sites of the hIL-3 gene. The codon choice used in the fragment corresponds to that found in highly expressed *E. coli* genes (Gouy and Gautier, 1982). Three new unique restriction sites, EcoRV, XhoI and PstI were introduced for the purpose of inserting synthetic gene fragments. The portion of the coding sequence shown encodes hIL-3 amino acids 20-70.

Figure 3 shows the nucleotide and amino acid sequence of the gene in pMON5873 with the sequence extending from NcoI through HindIII. The codon choices used to encode amino acids 1-14 and 107-133 correspond to that found in

highly expressed *E. coli* genes.

Figure 4 shows the construction of the plasmid vector pMON5846 which encodes [Met-(1-133) hIL-3 (Arg129)].

Figure 5 shows the construction of the plasmid vector pMON5847 (ATCC 68912) which encodes [Met-(1-133) hIL-3 (Arg129)].

Figure 6 shows the construction of plasmid vector pMON5853 which encodes [Met-(15-133) hIL-3 (Arg129)].

Figure 7 shows the construction of the plasmid vector pMON5854 which encodes [Met-(1-133) hIL-3 (Arg129)].

Figure 8 shows the DNA sequence and resulting amino acid sequence of the LamB signal peptide.

Figure 9 shows the construction of the plasmid vector pMON5978 which encodes Met-Ala-(15-125)hIL-3.

Figure 10 shows the construction of the plasmid vector pMON5988 which encodes Met-Ala(15-125)hIL-3.

Figure 11 shows the construction of the plasmid vector pMON5887 which encodes Met-(1-125)hIL-3.

Figure 12 shows the construction of pMON6457 which encodes (15-125)hIL-3; it contains the araBAD promoter and the LamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 13 shows the construction of pMON6458; it contains the araBAD promoter and the LamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 14 shows the construction of pMON13359.

Figure 15 shows the construction of pMON13352.

Figure 16 shows the construction of pMON13360.

Figure 17 shows the construction of pMON13363.

Figure 18 shows the construction of pMON13364.

Figure 19 shows the construction of pMON13365.

Figure 20 shows the construction of pMON13287.

Figure 21 shows the construction of pMON13288.

Figure 22 shows the construction of pMON13289.

Figure 23 shows the construction of pMON5723.

Figure 24 shows the construction of pMON13438.

Detailed Description of the Invention

The present invention relates to muteins of human interleukin-3 (hIL-3) in which amino acid substitutions have been made at four or more positions in amino acid sequence of the polypeptide and to muteins which have substantially the same structure and substantially the same biological activity. Preferred muteins of the present invention are (15-125)hIL-3 deletion mutants which have deletions of amino acids 1 to 14 at the N-terminus and 126 to 133 at the C-terminus and which also have four or more amino acid substitutions in the polypeptide and muteins having substantially the same structure and substantially the same biological activity. Among the preferred muteins are those having twenty-six amino acid substitutions. As used herein human interleukin-3 corresponds to the amino acid sequence (1-133) as depicted in Figure 1 and (15-125) hIL-3 corresponds to the 15 to 125 amino acid sequence of the hIL-3 polypeptide. Naturally occurring variants of hIL-3 polypeptide amino acids are also included in the present invention (for example, the allele in which proline rather than serine is at position 8 in the hIL-3 polypeptide sequence) as are variant hIL-3 molecules which are modified post-translationally (e.g. glycosylation).

The present invention also includes the DNA sequences which code for the mutant polypeptides, DNA sequences which are substantially similar and perform substantially the same function, and DNA sequences which differ from the DNAs encoding the muteins of the invention only due to the degeneracy of the genetic code.

Included in the present invention are novel mutant human interleukin-3 polypeptides comprising a polypeptide having the amino acid sequence of native human interleukin-3 wherein amino acids 126 to 133 have been deleted from the C-terminus of the native human interleukin-3 polypeptide and amino acids 1 to 14 have been deleted from the N-terminus of the native human interleukin-3 polypeptide and, in addition, polypeptides also have four or more amino acid substitutions in the polypeptide sequence.

Also included in the present invention are the DNA sequences coding for the muteins of the present invention; the oligonucleotide intermediates used to construct the mutant DNAs; and the polypeptides coded for by these oligonucleotides. These polypeptides may be useful as antagonists or as antigenic fragments for the production of antibodies useful in immunoassay and immunotherapy protocols.

The mutant hIL-3 polypeptides of the present invention may also have methionine, alanine, or methionine-alanine residues inserted at the N-terminus.

The present invention includes human interleukin-3 mutant polypeptide Formula I:

15

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn

1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

20

20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa

35 40 45

25

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

65 70 75

30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

80 85 90

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

35

95 100 105

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:15]

125

130

- 5 wherein Xaa at position 17 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;
- Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
- Xaa at position 19 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;
- Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;
- 10 Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;
- Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val or Gly;
- Xaa at position 23 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Leu, Ser, or Arg;
- 15 Xaa at position 24 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;
- Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
- Xaa at position 26 is His, Thr, Phe, Gly, Arg, Ala, or Trp;
- Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;
- 20 Xaa at position 28 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;
- Xaa at position 29 is Gln, Asn, Leu, Pro, Arg, or Val;
- Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;
- Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
- 25 Xaa at position 32 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;
- Xaa at position 33 is Pro, Leu, Gln, Ala, Thr, or Glu;
- Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr, Arg, Ala, Phe, Ile or Met;
- 30 Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;
- Xaa at position 36 is Asp, Leu, or Val;
- Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;
- Xaa at position 38 is Asn, or Ala;
- Xaa at position 40 is Leu, Trp, or Arg;
- 35 Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, or Pro;
- Xaa at position 42 is Gly, Asp, Ser, Cys, Asn, Lys, Thr, Leu, Val, Glu, Phe, Tyr, Ile, Met or Ala;
- Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys,

- Gln, Arg, Thr, Gly or Ser;  
Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp,  
Glu, Asn, Gln, Ala or Pro;  
Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys,  
5 Trp, Asp, Asn, Arg, Ser, Ala, Ile, Glu or His;  
Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln,  
Lys, His, Ala, Tyr, Ile, Val or Gly;  
Xaa at position 47 is Ile, Gly, Val, Ser, Arg, Pro, or His;  
Xaa at position 48 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu,  
10 Lys, Thr, Ala, Met, Val or Asn;  
Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;  
Xaa at position 50 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser,  
Ala, Ile, Val, His, Phe, Met or Gln;  
Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;  
15 Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;  
Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser,  
or Met;  
Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn,  
Lys, His, Ala or Leu;  
20 Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;  
Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His,  
Thr, Ala, Tyr, Phe, Leu, Val or Lys;  
Xaa at position 57 is Asn or Gly;  
Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;  
25 Xaa at position 59 is Glu Tyr, His, Leu, Pro, or Arg;  
Xaa at position 60 is Ala, Ser, Pro, Tyr, Asn, or Thr;  
Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;  
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, Asp, or Ile;  
Xaa at position 63 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or  
30 Val;  
Xaa at position 64 is Ala, Asn, Pro, Ser, or Lys;  
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;  
Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;  
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro,  
35 or His;  
Xaa at position 68 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or  
His;  
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly,

or Leu;

Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;

Xaa at position 71 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln,  
Trp, or Asn;

5 Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or  
Asp;

Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or  
Arg;

Xaa at position 74 is Ile, Met, Thr, Pro, Arg, Gly, Ala;

10 Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,  
Gln, or Leu;

Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly,  
or Asp;

Xaa at position 77 is Ile, Ser, Arg, Thr, or Leu;

15 Xaa at position 78 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;

Xaa at position 79 is Lys, Thr, Asn, Met, Arg, Ile, Gly, or  
Asp;

Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or  
Arg;

20 Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or  
Lys;

Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn,  
His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;

Xaa at position 83 is Pro, Ala, Thr, Trp, Arg, or Met;

25 Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;

Xaa at position 85 is Leu, Asn, Val, or Gln;

Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;

Xaa at position 87 is Leu, Ser, Trp, or Gly;

Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;

30 Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn,  
or Ser;

Xaa at position 90 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or  
Met;

Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;

35 Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile  
or Leu;

Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;

Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His,



Ala,

or Pro;

Xaa at position 95 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn,  
Lys,

5           Ser, Ala, Trp, Phe, Ile, or Tyr;

Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;

Xaa at position 97 is Ile, Val, Lys, Ala, or Asn;

Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr,  
Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;

10          Xaa at position 99 is Ile, Leu, Arg, Asp, Val, Pro, Gln,  
Gly, Ser, Phe, or His;

Xaa at position 100 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln,  
or Pro;

Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,

15           Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu, or Gln;

Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;

Xaa at position 103 is Asp, or Ser;

Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu,  
Gln, Lys, Ala, Phe, or Gly;

20          Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,  
Leu, Lys, Ile, Asp, or His;

Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;

Xaa at position 108 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser,  
Ala

25           or Pro;

Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;

Xaa at position 110 is Lys, Ala, Asn, Thr, Leu, Arg, Gln, His, Glu,  
Ser,

Ala, or Trp;

30          Xaa at position 111 is Leu, Ile, Arg, Asp, or Met;

Xaa at position 112 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;

Xaa at position 113 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp,  
Lys, Leu, Ile, Val or Asn;

Xaa at position 114 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;

35          Xaa at position 115 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr,  
Trp, or Met;

Xaa at position 116 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu,  
Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;

Xaa at position 117 is Thr, Ser, Asn, Ile, Trp, ~~Leu~~, or Pro;  
 Xaa at position 118 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;  
 Xaa at position 119 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;  
 Xaa at position 120 is Asn, Ala, Pro, Leu, His, Val, or Gln;  
 5 Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or  
 Gly;  
 Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,  
 Ile, Tyr, or Cys;  
 Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

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and which can additionally have Met- preceding the amino acid in  
 position 1; and wherein from 1 to 14 amino acids can be deleted from  
 the N-terminus and/or from 1 to 15 amino acids can be deleted from  
 the C-terminus; and wherein from 4 to 44 of the amino acids  
 15 designated by Xaa are different from the corresponding amino acids of  
 native (1-133) human interleukin-3.

Included in the present invention are human interleukin-3  
 mutant polypeptide of the Formula II:

20

Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	Lys	Thr	Ser	Trp	Val	Asn
1				5					10					15

25

Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa
				20					25					30

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	Leu	Xaa	Xaa	Glu	Xaa	Xaa
				35					40					45

30

Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	Leu	Xaa	Xaa
				50					55					60

35

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
				65					70					75

Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa
				80					85					90

5    Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa Xaa  
                        110                        115                        120

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15 Xaa at position 20 is Ile or Pro;

20 Xaa at position 26 is His, Phe, Gly, Arg, or Ala;

25 Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;

30 Xaa at position 36 is Asp or Leu;

35 Tyr, Val or Arg;

Xaa at position 45 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn, Glu, Ser, or Trp;

Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln, Glu,  
His, Ile, Lys, Tyr, Val or Gly;

Xaa at position 47 is Ile, Val, or His;

Xaa at position 49 is Met, Asn, or Asp;

5 Xaa at position 50 is Glu, Thr, Ala, Asn, Ser or Asp;

Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;

Xaa at position 52 is Asn or Gly;

Xaa at position 53 is Leu, Met, or Phe;

Xaa at position 54 is Arg, Ala, or Ser;

10 Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn, Glu,  
His,

Leu, Thr, Val or Lys;

Xaa at position 59 is Glu, Tyr, His, Leu, or Arg;

15 Xaa at position 60 is Ala, Ser, Asn, or Thr;

Xaa at position 61 is Phe or Ser;

Xaa at position 62 is Asn, Val, Pro, Thr, or Ile;

Xaa at position 63 is Arg, Tyr, Lys, Ser, His, or Val;

Xaa at position 64 is Ala or Asn;

20 Xaa at position 65 is Val, Thr, Leu, or Ser;

Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;

Xaa at position 67 is Ser, Phe, Val, Gly, Asn, Ile, or His;

Xaa at position 68 is Leu, Val, Ile, Phe, or His;

Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;

25 Xaa at position 70 is Asn or Pro;

Xaa at position 71 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;

Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;

Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or  
Pro;

30 Xaa at position 74 is Ile or Met;

Xaa at position 75 is Glu, Gly, Asp, Ser, or Gln;

Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or  
Asp;

Xaa at position 77 is Ile, Ser, or Leu;

35 Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or  
Asp;

Xaa at position 80 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;

Xaa at position 81 is Leu, or Val;

- Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His,  
Met, Phe, Ser, Thr, Tyr or Val;
- Xaa at position 83 is Pro, Ala, Thr, Trp, or Met;
- Xaa at position 85 is Leu or Val;
- 5 Xaa at position 87 is Leu or Ser;
- Xaa at position 88 is Ala, Arg, or Trp;
- Xaa at position 89 is Thr, Asp, Glu, His, Asn, or Ser;
- Xaa at position 90 is Ala, Asp, or Met;
- Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
- 10 Xaa at position 92 is Pro or Ser;
- Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
- Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe,  
Ser or Thr;
- Xaa at position 96 is Pro or Tyr;
- 15 Xaa at position 97 is Ile, Val, or Ala;
- Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Leu, Arg,  
Gln,  
Glu, Lys, Met, Ser, Tyr, Val or Pro;
- Xaa at position 99 is Ile, Leu, Val, or Phe;
- 20 Xaa at position 100 is Lys, Leu, His, Arg, Ile, Gln, Pro, or  
Ser;
- Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,  
Asn, Ile, Leu or Tyr;
- Xaa at position 102 is Gly, Glu, Lys, or Ser;
- 25 Xaa at position 104 is Trp, Val, Tyr, Met, or Leu;
- Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,  
Leu, Lys, Ile, Asp, or His;
- Xaa at position 106 is Glu, Ser, Ala, or Gly;
- Xaa at position 108 is Arg, Ala, Gln, Ser or Lys;
- 30 Xaa at position 109 is Arg, Thr, Glu, Leu, Ser, or Gly;
- Xaa at position 112 is Thr, Val, Gln, Glu, His, or Ser;
- Xaa at position 114 is Tyr or Trp;
- Xaa at position 115 is Leu or Ala;
- Xaa at position 116 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala, Asn,  
Gln, His, Met, Phe, Tyr or Ile;
- 35 Xaa at position 117 is Thr, Ser, or Asn;
- Xaa at position 119 is Glu, Ser, Pro, Leu, Thr, or Tyr;
- Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or Gly;

Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;

5 Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from  
 10 the C-terminus; and wherein from 4 to 44 of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3.

Included in the present invention are human interleukin-3  
 15 mutant polypeptide of the Formula III:

	Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	Lys	Thr	Ser	Trp	Val	Asn
	1				5					10					15
20	Cys	Xaa	Xaa	Xaa	Ile	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Leu	Lys	Xaa	Xaa
					20					25					30
	Xaa	Xaa	Xaa	Xaa	Xaa	Asp	Xaa	Xaa	Asn	Leu	Asn	Xaa	Glu	Xaa	Xaa
					35					40					45
25	Xaa	Ile	Leu	Met	Xaa	Xaa	Asn	Leu	Xaa	Xaa	Xaa	Asn	Leu	Glu	Xaa
					50					55					60
	Phe	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	Xaa	Xaa	Xaa	Ile	Glu
30					65					70					75
	Xaa	Xaa	Leu	Xaa	Xaa	Leu	Xaa	Xaa	Cys	Xaa	Pro	Xaa	Xaa	Thr	Ala
					80					85					90
35	Xaa	Pro	Xaa	Arg	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Gly	Asp	Xaa	Xaa
					95					100					105
	Xaa	Phe	Xaa	Xaa	Lys	Leu	Xaa	Phe	Xaa	Xaa	Xaa	Xaa	Leu	Glu	Xaa

110

115

120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:17]  
125 130

5

wherein

Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 18 is Asn, His, or Ile;

Xaa at position 19 is Met or Ile;

10 Xaa at position 21 is Asp or Glu;

Xaa at position 23 is Ile, Ala, Leu, or Gly;

Xaa at position 24 is Ile, Val, or Leu;

Xaa at position 25 is Thr, His, Gln, or Ala;

Xaa at position 26 is His or Ala;

15 Xaa at position 29 is Gln, Asn, or Val;

Xaa at position 30 is Pro, Gly, or Gln;

Xaa at position 31 is Pro, Asp, Gly, or Gln;

Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 33 is Pro or Glu;

20 Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,  
Glu, Ile, Phe, Thr or Met;

Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;

Xaa at position 37 is Phe, Ser, Pro, or Trp;

Xaa at position 38 is Asn or Ala;

25 Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,  
Met, Tyr or Arg;

Xaa at position 44 is Asp or Glu;

Xaa at position 45 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu,  
Ser or Lys;

30 Xaa at position 46 is Asp, Phe, Ser, Thr, Ala, Asn Gln, Glu, His,  
Ile, Lys, Tyr, Val or Cys;

Xaa at position 50 is Glu, Ala, Asn, Ser or Asp;

Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;

Xaa at position 54 is Arg or Ala;

35 Xaa at position 54 is Arg or Ala;

Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;

Xaa at position 56 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu,  
Leu, Thr, Val or Lys;

- Xaa at position 60 is Ala or Ser;  
Xaa at position 62 is Asn, Pro, Thr, or Ile;  
Xaa at position 63 is Arg or Lys;  
Xaa at position 64 is Ala or Asn;  
5 Xaa at position 65 is Val or Thr;  
Xaa at position 66 is Lys or Arg;  
Xaa at position 67 is Ser, Phe, or His;  
Xaa at position 68 is Leu, Ile, Phe, or His;  
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;  
10 Xaa at position 71 is Ala, Pro, or Arg;  
Xaa at position 72 is Ser, Glu, Arg, or Asp;  
Xaa at position 73 is Ala or Leu;  
Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;  
Xaa at position 77 is Ile or Leu;  
15 Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or  
Asp;  
Xaa at position 80 is Asn, Gly, Glu, or Arg;  
Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu, His,  
Ile, Met, Phe, Ser, Thr, Tyr or Val;  
20 Xaa at position 83 is Pro or Thr;  
Xaa at position 85 is Leu or Val;  
Xaa at position 87 is Leu or Ser;  
Xaa at position 88 is Ala or Trp;  
Xaa at position 91 is Ala or Pro;  
25 Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;  
Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe, Ser  
or Thr;  
Xaa at position 96 is Pro or Tyr;  
Xaa at position 97 is Ile or Val;  
30 Xaa at position 98 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg, Gln,  
Leu, Lys, Met, Ser, Tyr, Val or Pro;  
Xaa at position 99 is Ile, Leu, or Val;  
Xaa at position 100 is Lys, Arg, Ile, Gln, Pro, or Ser;  
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Pro, Asn,  
35 Ile, Leu or Tyr;  
Xaa at position 104 is Trp or Leu;  
Xaa at position 105 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu,  
Lys, Ile, Asp, or His;



Xaa at position 106 is Glu or Gly;

Xaa at position 108 is Arg, Ala, or Ser;

Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;

Xaa at position 112 is Thr, Val, or Gln;

5 Xaa at position 114 is Tyr or Trp;

Xaa at position 115 is Leu or Ala;

Xaa at position 116 is Lys, Thr, Val, Trp, Ser, Ala, His, Met,  
Phe, Tyr or Ile;

Xaa at position 117 is Thr or Ser;

10 Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;

Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,  
Ile, Tyr, or Cys;

Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

15

and which can additionally have Met- preceding the amino acid in  
position 1; and wherein from 1 to 14 amino acids can be deleted from  
the N-terminus and/or from 1 to 15 amino acids can be deleted from  
the C-terminus; and wherein from 4 to 35 of the amino acids

20 designated by Xaa are different from the corresponding amino acids of  
native (1-133)human interleukin-3.

Included in the present invention are human interleukin-3 mutant  
polypeptide of the Formula IV:

25

Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	Lys	Thr	Ser	Trp	Val	Asn
1				5					10					15

30

Cys	Xaa	Xaa	Met	Ile	Asp	Glu	Xaa	Ile	Xaa	Xaa	Leu	Lys	Xaa	Xaa
				20					25					30

Pro	Xaa	Pro	Xaa	Xaa	Asp	Phe	Xaa	Asn	Leu	Asn	Xaa	Glu	Asp	Xaa
				35					40					45

35

Xaa	Ile	Leu	Met	Xaa	Xaa	Asn	Leu	Arg	Xaa	Xaa	Asn	Leu	Glu	Ala
				50					55					60

Phe	Xaa	Arg	Xaa	Xaa	Lys	Xaa	Xaa	Xaa	Asn	Ala	Ser	Ala	Ile	Glu
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

65

70

75

Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr Ala  
80 85 90

5

Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp Xaa  
95 100 105

10

Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu Xaa  
110 115 120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID NO:18]  
125 130

wherein

- 15 Xaa at position 17 is Ser, Gly, Asp, or Gln;  
Xaa at position 18 is Asn, His, or Ile;  
Xaa at position 23 is Ile, Ala, Leu, or Gly;  
Xaa at position 25 is Thr, His, or Gln;  
Xaa at position 26 is His or Ala;
- 20 Xaa at position 29 is Gln or Asn;  
Xaa at position 30 is Pro or Gly;  
Xaa at position 32 is Leu, Arg, Asn, or Ala;  
Xaa at position 34 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile,  
Phe, Thr, or Met;
- 25 Xaa at position 35 is Leu, Ala, Asn, or Pro;  
Xaa at position 38 is Asn or Ala;  
Xaa at position 42 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met,  
Tyr or Arg;
- 30 Xaa at position 45 is Gln, Val, Met, Leu, Ala, Asn, Glu, or Lys;  
Xaa at position 46 is Asp, Phe, Ser, Gln, Glu, His, Val  
or Thr;
- Xaa at position 50 is Glu Asn, Ser or Asp;  
Xaa at position 51 is Asn, Arg, Pro, Thr, or His;  
Xaa at position 55 is Arg, Leu, or Gly;
- 35 Xaa at position 56 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;  
Xaa at position 62 is Asn, Pro, or Thr;  
Xaa at position 64 is Ala or Asn;  
Xaa at position 65 is Val or Thr;

- Xaa at position 67 is Ser or Phe;  
 Xaa at position 68 is Leu or Phe;  
 Xaa at position 69 is Gln, Ala, Glu, or Arg;  
 Xaa at position 76 is Ser, Val, Asn, Pro, or Gly;  
 5 Xaa at position 77 is Ile or Leu;  
 Xaa at position 79 is Lys, Gly, Asn, Met, Arg, Ile, or Gly;  
 Xaa at position 80 is Asn, Gly, Glu, or Arg;  
 Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His, Met,  
     Phe, Ser, Thr, Tyr or Val;  
 10 Xaa at position 87 is Leu or Ser;  
 Xaa at position 88 is Ala or Trp;  
 Xaa at position 91 is Ala or Pro;  
 Xaa at position 93 is Thr, Asp, or Ala;  
 Xaa at position 95 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;  
 15 Xaa at position 98 is His, Ile, Asn, Ala, Thr, Gln, Glu,  
     Lys, Met, Ser, Tyr, Val or Leu;  
 Xaa at position 99 is Ile or Leu;  
 Xaa at position 100 is Lys or Arg;  
 Xaa at position 101 is Asp, Pro, Met, Lys, Thr, His, Pro, Asn, Ile,  
 20 Leu or Tyr;  
 Xaa at position 105 is Asn, Pro, Ser, Ile or Asp;  
 Xaa at position 108 is Arg, Ala, or Ser;  
 Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;  
 Xaa at position 112 is Thr or Gln;  
 25 Xaa at position 116 is Lys, Val, Trp, Ala, His, Phe, Tyr or Ile;  
 Xaa at position 117 is Thr or Ser;  
 Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;  
 Xaa at position 121 is Ala, Ser, Ile, Pro, or Asp;  
 Xaa at position 122 is Gln, Met, Trp, Phe, Pro, His, Ile, or Tyr;  
 30 Xaa at position 123 is Ala, Met, Glu, Ser, or Leu;
- and which can additionally have Met- preceding the amino acid in  
 position 1; and wherein from 1 to 14 amino acids can be deleted from  
 the N-terminus and/or from 1 to 15 amino acids can be deleted from  
 35 the C-terminus; and wherein from 4 to 44 of the amino acids  
 designated by Xaa are different from the corresponding amino acids of  
 native (1-133)human interleukin-3.

Included in the present invention are (15-125)human  
interleukin-3 mutant polypeptides of the Formula V:

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5  Asn Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    1      5      10      15

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa
        20      25      30

10  Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35      40      45

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        50      55      60

15  Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        65      70      75

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        80      85      90

20  Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        95      100     105

25  Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:19]
        110

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wherein

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30  Xaa at position 3 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;
    Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
    Xaa at position 5 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;
    Xaa at position 6 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;
    Xaa at position 7 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn,
        Thr, Ser or Val;

35  Xaa at position 8 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln,
        Leu, Val, or Gly;
    Xaa at position 9 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe,
        Leu, Ser, or Arg;

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- Xaa at position 10 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;  
Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;  
Xaa at position 12 is His, Thr, Phe, Gly, Arg, Ala, or Trp;  
Xaa at position 13 is Leu, Gly, Arg, Thr, Ser, or Ala;  
5 Xaa at position 14 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;  
Xaa at position 15 is Gln, Asn, Leu, Pro, Arg, or Val;  
Xaa at position 16 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or  
Lys;  
Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;  
10 Xaa at position 18 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;  
Xaa at position 19 is Pro, Leu, Gln, Ala, Thr, or Glu;  
Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr,  
Arg, Ala, Phe, Ile or Met;  
Xaa at position 21 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;  
15 Xaa at position 22 is Asp, Leu, or Val;  
Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;  
Xaa at position 24 is Asn, or Ala;  
Xaa at position 26 is Leu, Trp, or Arg;  
Xaa at position 27 is Asn, Cys, Arg, Leu, His, Met, Pro;  
20 Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Lys, Asn, Thr, Leu,  
Val, Glu, Phe, Tyr, Ile or Met;  
Xaa at position 29 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln,  
Arg, Thr, Gly or Ser;  
Xaa at position 30 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu,  
25 Asn, Gln, Ala or Pro;  
Xaa at position 31 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Asp,  
Asn, Arg, Ser, Ala, Ile, Glu, His or Trp;  
Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln,  
Lys, His, Ala, Tyr, Ile, Val or Gly;  
30 Xaa at position 33 is Ile, Gly, Val, Ser, Arg, Pro, or His;  
Xaa at position 34 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu,  
Lys, Thr, Ala, Met, Val or Asn;  
Xaa at position 35 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;  
Xaa at position 36 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala,  
35 Ile, Val, His, Phe, Met or Gln;  
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;  
Xaa at position 38 is Asn, His, Arg, Leu, Gly, Ser, or Thr;  
Xaa at position 39 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser,

Met, or;

Xaa at position 40 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn,  
Lys, His, Ala or Leu;

Xaa at position 41 is Arg, Thr, Val, Ser, Leu, or Gly;

5 Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His,  
Thr, Ala, Tyr, Phe, Leu, Val or Lys;

Xaa at position 43 is Asn or Gly;

Xaa at position 44 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;

Xaa at position 45 is Glu Tyr, His, Leu, Pro, or Arg;

10 Xaa at position 46 is Ala, Ser, Pro, Tyr, Asn, or Thr;

Xaa at position 47 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;

Xaa at position 48 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;

Xaa at position 49 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;

Xaa at position 50 is Ala, Asn, Pro, Ser, or Lys;

15 Xaa at position 51 is Val, Thr, Pro, His, Leu, Phe, or Ser;

Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;

Xaa at position 53 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or  
His;

Xaa at position 54 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;

20 Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or  
Leu;

Xaa at position 56 is Asn, Leu, Val, Trp, Pro, or Ala;

Xaa at position 57 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln,  
Trp, or Asn;

25 Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;

Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;

Xaa at position 60 is Ile, Met, Thr, Pro, Arg, Gly, Ala;

Xaa at position 61 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,  
Gln, or Leu;

30 Xaa at position 62 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or  
Asp;

Xaa at position 63 is Ile, Ser, Arg, Thr, or Leu;

Xaa at position 64 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;

Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or

35 Asp;

Xaa at position 66 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;

Xaa at position 67 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;

Xaa at position 68 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn,

- His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
- Xaa at position 69 is Pro, Ala, Thr, Trp, Arg, or Met;
- Xaa at position 70 is Cys, Glu, Gly, Arg, Met, or Val;
- Xaa at position 71 is Leu, Asn, Val, or Gln;
- 5 Xaa at position 72 is Pro, Cys, Arg, Ala, or Lys;
- Xaa at position 73 is Leu, Ser, Trp, or Gly;
- Xaa at position 74 is Ala, Lys, Arg, Val, or Trp;
- Xaa at position 75 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;
- 10 Xaa at position 76 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;
- Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
- Xaa at position 78 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile or Leu;
- Xaa at position 79 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
- 15 Xaa at position 80 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys, His, Ala or Pro;
- Xaa at position 81 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr, Asn, Lys, Ser, Ala, Trp, Phe, Ile or Tyr;
- Xaa at position 82 is Pro, Lys, Tyr, Gly, Ile, or Thr;
- 20 Xaa at position 83 is Ile, Val, Lys, Ala, or Asn;
- Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
- Xaa at position 85 is Ile, Leu, Arg, Asp, Val, Pro, Gln, Gly, Ser, Phe, or His;
- 25 Xaa at position 86 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln, Pro;
- Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val, Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu or Gln;
- Xaa at position 88 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
- 30 Xaa at position 89 is Asp, or Ser;
- Xaa at position 90 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu, Gln, Lys, Ala, Phe, or Gly;
- Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr, Leu, Lys, Ile, Asp, or His;
- 35 Xaa at position 92 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;
- Xaa at position 94 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His, Ser, Ala, or Pro;
- Xaa at position 95 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;

Xaa at position 96 is Lys, Asn, Thr, Leu, Gln, Arg,

His, Glu, Ser, Ala or Trp;

Xaa at position 97 is Leu, Ile, Arg, Asp, or Met;

Xaa at position 98 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;

5 Xaa at position 99 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp,

Lys, Leu, Ile, Val or Asn;

Xaa at position 100 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;

Xaa at position 101 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr,

Trp, or Met;

10 Xaa at position 102 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu, Arg,  
Trp,

Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;

Xaa at position 103 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;

Xaa at position 104 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;

15 Xaa at position 105 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;

Xaa at position 106 is Asn, Ala, Pro, Leu, His, Val, or Gln;

Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or

Gly;

Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,

20 Ile, Tyr, or Cys;

Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- or Met-Ala- preceding the amino  
acid in position 1; and wherein from 4 to 44 of the amino acids

25 designated by Xaa are different from the corresponding native amino  
acids of (1-133) human interleukin-3; or a polypeptide having  
substantially the same structure and substantially the same  
biological activity.

30 Included in the present invention are (15-125) human  
interleukin-3 mutant polypeptides of the Formula VI:

Asn	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa
1				5					10				15	

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	Leu	Xaa	Xaa	Glu	Xaa
									20		25		30	





- Xaa at position 22 is Asp or Leu;  
Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;  
Xaa at position 24 is Asn or Ala;  
Xaa at position 27 is Asn, Cys, Arg, His, Met, or Pro;  
5 Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,  
Met, Tyr, or Arg;  
Xaa at position 30 is Asp, or Glu;  
Xaa at position 31 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn Glu,  
Ser or Trp;  
10 Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln,  
Glu, His, Ile, Lys, Tyr, Val or Gly;  
Xaa at position 33 is Ile, Val, or His;  
Xaa at position 35 is Met, Asn, or Asp;  
Xaa at position 36 is Glu, Thr, Ala, Asn, Ser or Asp;  
15 Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;  
Xaa at position 38 is Asn or Gly;  
Xaa at position 39 is Leu, Met, or Phe;  
Xaa at position 40 is Arg, Ala or Ser;  
Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;  
20 Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn,  
Glu, His, Leu, Thr, Val or Lys;  
Xaa at position 45 is Glu, Tyr, His, Leu, or Arg;  
Xaa at position 46 is Ala, Ser, Asn, or Thr;  
Xaa at position 47 is Phe or Ser;  
25 Xaa at position 48 is Asn, Val, Pro, Thr, or Ile;  
Xaa at position 49 is Arg, Tyr, Lys, Ser, His, or Val;  
Xaa at position 50 is Ala or Asn;  
Xaa at position 51 is Val, Thr, Leu, or Ser;  
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;  
30 Xaa at position 53 is Ser, Phe, Val, Gly, Asn, Ile, or His;  
Xaa at position 54 is Leu, Val, Ile, Phe, or His;  
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;  
Xaa at position 56 is Asn or Pro;  
Xaa at position 57 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;  
35 Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;  
Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or  
Pro;  
Xaa at position 60 is Ile or Met;

- Xaa at position 61 is Glu, Gly, Asp, Ser, or Gln;  
Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or  
Asp;  
Xaa at position 63 is Ile, Ser, or Leu;  
5 Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or  
Asp;  
Xaa at position 66 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;  
Xaa at position 67 is Leu, or Val;  
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,  
10 His, Met, Phe, Ser, Thr, Tyr or Val;  
Xaa at position 69 is Pro, Ala, Thr, Trp, or Met;  
Xaa at position 71 is Leu or Val;  
Xaa at position 73 is Leu or Ser;  
Xaa at position 74 is Ala, Arg, or Trp;  
15 Xaa at position 75 is Thr, Asp, Glu, His, Asn, or Ser;  
Xaa at position 76 is Ala, Asp, or Met;  
Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;  
Xaa at position 78 is Pro or Ser;  
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;  
20 Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile, Phe,  
Ser or Thr;  
Xaa at position 82 is Pro or Tyr;  
Xaa at position 83 is Ile, Val, or Ala;  
Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr,  
25 Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Pro;  
Xaa at position 85 is Ile, Leu, Val, or Phe;  
Xaa at position 86 is Lys, Leu, His, Arg, Ile, Gln, Pro or  
Ser;  
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val,  
30 Asn, Ile, Leu or Tyr;  
Xaa at position 88 is Gly, Glu, Lys, or Ser;  
Xaa at position 90 is Trp, Val, Tyr, Met, or Leu;  
Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,  
Leu, Lys, Ile, Asp, or His;  
35 Xaa at position 92 is Glu, Ser, Ala, or Gly;  
Xaa at position 94 is Arg, Ala, Gln, Ser or Lys;  
Xaa at position 95 is Arg, Thr, Glu, Leu, Ser, or Gly;  
Xaa at position 98 is Thr, Val, Gln, Glu, His, or Ser;

Xaa at position 100 is Tyr or Trp;

Xaa at position 101 is Leu or Ala;

Xaa at position 102 is Lys, Thr, Met, Val, Trp, Ser, Leu,

Ala, Asn, Gln, His, Met, Phe, Tyr or Ile;

5 Xaa at position 103 is Thr, Ser, or Asn;

Xaa at position 105 is Glu, Ser, Pro, Leu, Thr, or Tyr;

Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or

Gly;

10 Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,

Ile, Tyr, or Cys;

Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

15 and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from 4 to 44 of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

20

Included in the present invention are (15-125)human interleukin-3 mutant polypeptides of the Formula VII:

25	Asn Cys Xaa Xaa Xaa Ile Xaa Glu Xaa Xaa Xaa Xaa Leu Lys Xaa	1	5	10	15
	Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Leu Asn Xaa Glu Xaa	20	25	30	
30	Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Xaa Xaa Xaa Asn Leu Glu	35	40	45	
	Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Ile	50	55	60	
35	Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr	65	70	75	

Ala Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gln Asp Xaa

80

85

90

Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu

5

95

100

105

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:21]

110

10 wherein

Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;

Xaa at position 4 is Asn, His, or Ile;

Xaa at position 5 is Met or Ile;

Xaa at position 7 is Asp or Glu;

15 Xaa at position 9 is Ile, Ala, Leu, or Gly;

Xaa at position 10 is Ile, Val, or Leu;

Xaa at position 11 is Thr, His, Gln, or Ala;

Xaa at position 12 is His or Ala;

Xaa at position 15 is Gln, Asn, or Val;

20 Xaa at position 16 is Pro, Gly, or Gln;

Xaa at position 17 is Pro, Asp, Gly, or Gln;

Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 19 is Pro or Glu;

Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg,

25 Gln, Glu, Ile, Phe, Thr or Met;

Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;

Xaa at position 23 is Phe, Ser, Pro, or Trp;

Xaa at position 24 is Asn or Ala;

Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile,

30 Leu, Met Tyr or Arg;

Xaa at position 30 is Asp or Glu;

Xaa at position 31 is Gln, Val, Met, Leu, Thr, Ala, Asn,

Glu, Ser or Lys;

Xaa at position 32 is Asp, Phe, Ser, Thr, Ala, Asn, Gln, Glu,

35 His, Ile, Lys, Tyr, Val or Cys;

Xaa at position 36 is Glu, Ala, Asn, Ser or Asp;

Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;

Xaa at position 40 is Arg or Ala;

- Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;  
Xaa at position 42 is Pro, Gly, Ser, Gln, Ala, Arg, Asn,  
Glu, Leu, Thr, Val or Lys;  
Xaa at position 46 is Ala or Ser;  
5 Xaa at position 48 is Asn, Pro, Thr, or Ile;  
Xaa at position 49 is Arg or Lys;  
Xaa at position 50 is Ala or Asn;  
Xaa at position 51 is Val or Thr;  
Xaa at position 52 is Lys or Arg;  
10 Xaa at position 53 is Ser, Phe, or His;  
Xaa at position 54 is Leu, Ile, Phe, or His;  
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;  
Xaa at position 57 is Ala, Pro, or Arg;  
Xaa at position 58 is Ser, Glu, Arg, or Asp;  
15 Xaa at position 59 is Ala or Leu;  
Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;  
Xaa at position 63 is Ile or Leu;  
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly,  
or Asp;  
20 Xaa at position 66 is Asn, Gly, Glu, or Arg;  
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,  
His, Ile, Met, Phe, Ser, Thr, Tyr or Val;  
Xaa at position 69 is Pro or Thr;  
Xaa at position 71 is Leu or Val;  
25 Xaa at position 73 is Leu or Ser;  
Xaa at position 74 is Ala or Trp;  
Xaa at position 77 is Ala or Pro;  
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;  
Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe,  
30 Ser or Thr;  
Xaa at position 82 is Pro or Tyr;  
Xaa at position 83 is Ile or Val;  
Xaa at position 84 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg,  
Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;  
35 Xaa at position 85 is Ile, Leu, or Val;  
Xaa at position 86 is Lys, Arg, Ile, Gln, Pro, or Ser;  
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Asn, Ile,  
Leu or Tyr;

- Xaa at position 90 is Trp or Leu;  
 Xaa at position 91 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu,  
 Lys, Ile, Asp, or His;  
 Xaa at position 92 is Glu, or Gly;  
 5 Xaa at position 94 is Arg, Ala, or Ser;  
 Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;  
 Xaa at position 98 is Thr, Val, or Gln;  
 Xaa at position 100 is Tyr or Trp;  
 Xaa at position 101 is Leu or Ala;  
 10 Xaa at position 102 is Lys, Thr, Val, Trp, Ser, Ala, His,  
 Met, Phe, Tyr or Ile;  
 Xaa at position 103 is Thr or Ser;  
 Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;  
 Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;  
 15 Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,  
 Ile, Tyr, or Cys;  
 Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

- which can additionally have Met- or Met-Ala- preceding the amino acid  
 20 in position 1; and wherein from 4 to 35 of the amino acids designated  
 by Xaa are different from the corresponding amino acids of native  
 human interleukin-3.

- 25 Included in the present invention are (15-125)human  
 interleukin-3 mutant polypeptides of the Formula VIII:

	Asn	Cys	Xaa	Xaa	Met	Ile	Asp	Glu	Xaa	Ile	Xaa	Xaa	Leu	Lys	Xaa
	1				5					10				15	
30	Xaa	Pro	Xaa	Pro	Xaa	Xaa	Asp	Phe	Xaa	Asn	Leu	Asn	Xaa	Glu	Asp
					20					25				30	
	Xaa	Xaa	Ile	Leu	Met	Xaa	Xaa	Asn	Leu	Arg	Xaa	Xaa	Asn	Leu	Glu
35					35					40				45	
	Ala	Phe	Xaa	Arg	Xaa	Xaa	Lys	Xaa	Xaa	Xaa	Asn	Ala	Ser	Ala	Ile
					50					55				60	

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr  
65 70 75

5 Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Gly Asp Trp  
80 85 90

Xaa Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu  
95 100 105

10

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:22]  
110

wherein

- 15 Xaa at position 3 is Ser, Gly, Asp, or Gln;  
Xaa at position 4 is Asn, His, or Ile;  
Xaa at position 9 is Ile, Ala, Leu, or Gly;  
Xaa at position 11 is Thr, His, or Gln;  
Xaa at position 12 is His or Ala;
- 20 Xaa at position 15 is Gln or Asn;  
Xaa at position 16 is Pro or Gly;  
Xaa at position 18 is Leu, Arg, Asn, or Ala;  
Xaa at position 20 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile,  
Phe, Thr or Met;
- 25 Xaa at position 21 is Leu, Ala, Asn, or Pro;  
Xaa at position 24 is Asn or Ala;  
Xaa at position 28 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met,  
Tyr or Arg;
- Xaa at position 31 is Gln, Val, Met, Leu, Ala, Asn, Glu or Lys;
- 30 Xaa at position 32 is Asp, Phe, Ser, Ala, Gln, Glu, His, Val  
or Thr;
- Xaa at position 36 is Glu, Asn, Ser or Asp;  
Xaa at position 37 is Asn, Arg, Pro, Thr, or His;  
Xaa at position 41 is Arg, Leu, or Gly;
- 35 Xaa at position 42 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;  
Xaa at position 48 is Asn, Pro, or Thr;  
Xaa at position 50 is Ala or Asn;  
Xaa at position 51 is Val or Thr;



- Xaa at position 53 is Ser or Phe;  
 Xaa at position 54 is Leu or Phe;  
 Xaa at position 55 is Gln, Ala, Glu, or Arg;  
 Xaa at position 62 is Ser, Val, Asn, Pro, or Gly;  
 5 Xaa at position 63 is Ile or Leu;  
 Xaa at position 65 is Lys, Asn, Met, Arg, Ile, or Gly;  
 Xaa at position 66 is Asn, Gly, Glu, or Arg;  
 Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His,  
     Met, Phe, Ser, Thr, Tyr or Val;  
 10 Xaa at position 73 is Leu or Ser;  
 Xaa at position 74 is Ala or Trp;  
 Xaa at position 77 is Ala or Pro;  
 Xaa at position 79 is Thr, Asp, or Ala;  
 Xaa at position 81 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;  
 15 Xaa at position 84 is His, Ile, Asn, Ala, Thr, Arg, Gln, Glu,  
     Lys, Met, Ser, Tyr, Val or Leu;  
 Xaa at position 85 is Ile or Leu;  
 Xaa at position 86 is Lys or Arg;  
 Xaa at position 87 is Asp, Pro, Met, Lys, His, Pro, Asn, Ile,  
 20 Leu or Tyr;  
 Xaa at position 91 is Asn, Pro, Ser, Ile or Asp;  
 Xaa at position 94 is Arg, Ala, or Ser;  
 Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;  
 Xaa at position 98 is Thr or Gln;  
 25 Xaa at position 102 is Lys, Val, Trp, or Ile;  
 Xaa at position 103 is Thr, Ala, His, Phe, Tyr or Ser;  
 Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;  
 Xaa at position 107 is Ala, Ser, Ile, Pro, or Asp;  
 Xaa at position 108 is Gln, Met, Trp, Phe, Pro, His, Ile, or  
 30 Tyr;  
 Xaa at position 109 is Ala, Met, Glu, Ser, or Leu;

- and which can additionally have Met- or Met-Ala- preceding the amino  
 acid in position 1; and wherein from 4 to 26 of the amino acids  
 35 designated by Xaa are different from the corresponding amino acids of  
 native (1-133)human interleukin-3; or a polypeptide having  
 substantially the same structure and substantially the same  
 biological activity.

The present invention includes polypeptides of the formula

	1		5		10
5	(Met) <sub>m</sub> -Ala	Pro	Met	Thr	Gln Thr Thr Ser Leu Lys Thr
	15		20		
	Ser Trp Val Asn Cys Ser Xaa Xaa Xaa Asp Glu Ile Ile				
25		30		35	
	Xaa His Leu Lys Xaa Pro Pro Xaa Pro Xaa Leu Asp Xaa				
10	40		45		50
	Xaa Asn Leu Asn Xaa Glu Asp Xaa Asp Ile Leu Xaa Glu				
	55		60		
	Xaa Asn Leu Arg Xaa Xaa Asn Leu Xaa Xaa Phe Xaa Xaa				
	65		70		75
15	Ala Xaa Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa				
	80		85		
	Ile Leu Xaa Asn Leu Xaa Pro Cys Xaa Pro Xaa Xaa Thr				
90		95		100	
	Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Ile Xaa Xaa Gly				
20	105		110		115
	Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Xaa Phe Tyr Leu				
	120		125		
	Xaa Xaa Leu Glu Xaa Ala Gln Xaa Gln Gln Thr Thr Leu				
	130				
25	Ser Leu Ala Ile Phe [SEQ ID NO:129]				

wherein m is 0 or 1; Xaa at position 18 is Asn or Ile; Xaa at position 19 is Met, Ala or Ile; Xaa at position 20 is Ile, Pro or Ile; Xaa at position 23 is Ile, Ala or Leu; Xaa at position 25 is Thr or His; Xaa at position 29 is Gln, Arg, Val or Ile; Xaa at position 32 is Leu, Ala, Asn or Arg; Xaa at position 34 is Leu or Ser; Xaa at position 37 is Phe, Pro, or Ser; Xaa at position 38 is Asn or Ala; Xaa at position 42 is Gly, Ala, Ser, Asp or Asn; Xaa at position 45 is Gln, Val, or Met; Xaa at position 46 is Asp or Ser; Xaa at position 49 is Met, Ile, Leu or Asp; Xaa at position 50 is Glu or Asp; Xaa at position 51 is Asn Arg or Ser; Xaa at position 55 is Arg, Leu, or Thr; Xaa at

position 56 is Pro or Ser; Xaa at position 59 is Glu or  
 Leu; Xaa at position 60 is Ala or Ser; Xaa at position 62  
 is Asn, Val or Pro; Xaa at position 63 is Arg or His; Xaa  
 at position 65 is Val or Ser; Xaa at position 67 is Ser,  
 5 Asn, His or Gln; Xaa at position 69 is Gln or Glu; Xaa at  
 position 73 is Ala or Gly; Xaa at position 76 is Ser, Ala  
 or Pro; Xaa at position 79 is Lys, Arg or Ser; Xaa at  
 position 82 is Leu, Glu, Val or Trp; Xaa at position 85 is  
 Leu or Val; Xaa at position 87 is Leu, Ser, Tyr; Xaa at  
 10 position 88 is Ala or Trp; Xaa at position 91 is Ala or  
 Pro; Xaa at position 93 is Pro or Ser; Xaa at position 95  
 is His or Thr; Xaa at position 98 is His, Ile, or Thr; Xaa  
 at position 100 is Lys or Arg; Xaa at position 101 is Asp,  
 Ala or Met; Xaa at position 105 is Asn or Glu; Xaa at  
 15 position 109 is Arg, Glu or Leu; Xaa at position 112 is Thr  
 or Gln; Xaa at position 116 is Lys, Val, Trp or Ser; Xaa at  
 position 117 is Thr or Ser; Xaa at position 120 is Asn,  
 Gln, or His; Xaa at position 123 is Ala or Glu; with the  
 proviso that from four to twenty-six of the amino acids  
 20 designated by Xaa are different from the corresponding  
 amino acids of native human interleukin-3; or a polypeptide  
 having substantially the same structure and substantially  
 the same biological activity.

Preferred polypeptides of the present invention are  
 25 those of the formula

	1	5	10
	(Met <sub>m</sub> -Alan) <sub>p</sub> -Asn	Cys Ser Xaa Xaa Xaa Asp	Glu Xaa Ile
	15	20	
	Xaa His Leu Lys Xaa Pro Pro Xaa Pro Xaa Leu Asp Xaa		
30	25	30	35
	Xaa Asn Leu Asn Xaa Glu Asp Xaa Xaa Ile Leu Xaa Glu		
	40	45	
	Xaa Asn Leu Arg Xaa Xaa Asn Leu Xaa Xaa Phe Xaa Xaa		
	50	55	60
35	Ala Xaa Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa		
	65	70	75
	Ile Leu Xaa Asn Xaa Xaa Pro Cys Xaa Pro Xaa Ala Thr		
	80	85	

Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Ile Xaa Xaa Gly

90

95

100

Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Xaa Phe Tyr Leu

105

110

5 Xaa Xaa Leu Glu Xaa Ala Gln Xaa Gln Gln [SEQ ID NO:130]

wherein m is 0 or 1; n is 0 or 1; p is 0 or 1; Xaa at  
 position 4 is Asn or Ile; Xaa at position 5 is Met, Ala or  
 Ile; Xaa at position 6 is Ile, Pro or Leu; Xaa at position  
 10 9 is Ile, Ala or Leu; Xaa at position 11 is Thr or His; Xaa  
 at position 15 is Gln, Arg, Val or Ile; Xaa at position 18  
 is Leu, Ala, Asn or Arg; Xaa at position 20 is Leu or Ser;  
 Xaa at position 23 is Phe, Pro, or Ser; Xaa at position 24  
 is Asn or Ala; Xaa at position 28 is Gly, Ala, Ser, Asp or  
 15 Asn; Xaa at position 31 is Gln, Val, or Met; Xaa at  
 position 32 is Asp or Ser; Xaa at position 35 is Met, Ile  
 or Asp; Xaa at position 36 is Glu or Asp; Xaa at position  
 37 is Asn, Arg or Ser; Xaa at position 41 is Arg, Leu, or  
 Thr; Xaa at position 42 is Pro or Ser; Xaa at position 45  
 20 is Glu or Leu; Xaa at position 46 is Ala or Ser; Xaa at  
 position 48 is Asn, Val or Pro; Xaa at position 49 is Arg  
 or His; Xaa at position 51 is Val or Ser; Xaa at position  
 53 is Ser, Asn, His or Gln; Xaa at position 55 is Gln or  
 Glu; Xaa at position 59 is Ala or Gly; Xaa at position 62  
 25 is Ser, Ala or Pro; Xaa at position 65 is Lys, Arg or Ser;  
 Xaa at position 67 is Leu, Glu, or Val; Xaa at position 68  
 is Leu, Glu, Val or Trp; Xaa at position 71 is Leu or Val;  
 Xaa at position 73 is Leu, Ser or Tyr; Xaa at position 74  
 is Ala or Trp; Xaa at position 77 is Ala or Pro; Xaa at  
 30 position 79 is Pro or Ser; Xaa at position 81 is His or  
 Thr; Xaa at position 84 is His, Ile, or Thr; Xaa at  
 position 86 is Lys or Arg; Xaa at position 87 is Asp, Ala  
 or Met; Xaa at position 91 is Asn or Glu; Xaa at position  
 95 is Arg, Glu, Leu; Xaa at position 98 Thr or Gln; Xaa at  
 35 position 102 is Lys, Val, Trp or Ser; Xaa at position 103  
 is Thr or Ser; Xaa at position 106 is Asn, Gln, or His; Xaa  
 at position 109 is Ala or Glu; with the proviso that from  
 four to twenty-six of the amino acids designated by Xaa are

different from the corresponding amino acids of native (15-125)human interleukin-3; or a polypeptide having substantially the same structure and substantially the same biological activity.

5 "Mutant amino acid sequence," "mutant protein" or "mutant polypeptide" refers to a polypeptide having an amino acid sequence which varies from a native sequence or is encoded by a nucleotide sequence intentionally made variant from a native sequence. "Mutant protein," "variant  
10 protein" or "mutein" means a protein comprising a mutant amino acid sequence and includes polypeptides which differ from the amino acid sequence of native hIL-3 due to amino acid deletions, substitutions, or both. "Native sequence" refers to an amino acid or nucleic acid sequence which is  
15 identical to a wild-type or native form of a gene or protein.

Human IL-3 can be characterized by its ability to stimulate colony formation by human hematopoietic progenitor cells. The colonies formed include erythroid,  
20 granulocyte, megakaryocyte, granulocytic macrophages and mixtures thereof. Human IL-3 has demonstrated an ability to restore bone marrow function and peripheral blood cell populations to therapeutically beneficial levels in studies performed initially in primates and subsequently in humans  
25 (Gillio, A. P., et al. (1990); Ganser, A, et al. (1990); Falk, S., et al. (1991). Additional activities of hIL-3 include the ability to stimulate leukocyte migration and chemotaxis; the ability to prime human leukocytes to produce high levels of inflammatory mediators like  
30 leukotrienes and histamine; the ability to induce cell surface expression of molecules needed for leukocyte adhesion; and the ability to trigger dermal inflammatory responses and fever. Many or all of these biological activities of hIL-3 involve signal transduction and high  
35 affinity receptor binding. Mutant polypeptides of the present invention may exhibit useful properties such as having similar or greater biological activity when compared to native hIL-3 or by having improved half-life or

decreased adverse side effects, or a combination of these properties. They may also be useful as antagonists. hIL-3 mutant polypeptides which have little or no activity when compared to native hIL-3 may still be useful as

5 antagonists, as antigens for the production of antibodies for use in immunology or immunotherapy, as genetic probes or as intermediates used to construct other useful hIL-3 muteins. Since hIL-3 functions by binding to its

10 receptor(s) and triggering second messages resulting in competent signal transduction, hIL-3 muteins of this invention may be useful in helping to determine which specific amino acid sequences are responsible for these activities.

The novel hIL-3 mutant polypeptides of the present

15 invention will preferably have at least one biological property of human IL-3 or of an IL-3-like growth factor and may have more than one IL-3-like biological property, or an improved property, or a reduction in an undesirable biological property of human IL-3. Some mutant

20 polypeptides of the present invention may also exhibit an improved side effect profile. For example, they may exhibit a decrease in leukotriene release or histamine release when compared to native hIL-3 or (15-125) hIL-3. Such hIL-3 or hIL-3-like biological properties may include

25 one or more of the following biological characteristics and in vivo and in vitro activities.

One such property is the support of the growth and differentiation of progenitor cells committed to erythroid, lymphoid, and myeloid lineages. For example, in a standard

30 human bone marrow assay, an IL-3-like biological property is the stimulation of granulocytic type colonies, megakaryocytic type colonies, monocyte/macrophage type colonies, and erythroid bursts. Other IL-3-like properties are the interaction with early multipotential stem cells,

35 the sustaining of the growth of pluripotent precursor cells, the ability to stimulate chronic myelogenous leukemia (CML) cell proliferation, the stimulation of proliferation of mast cells, the ability to support the

growth of various factor-dependent cell lines, and the ability to trigger immature bone marrow cell progenitors. Other biological properties of IL-3 have been disclosed in the art. Human IL-3 also has some biological activities which may in some cases be undesirable, for example the ability to stimulate leukotriene release and the ability to stimulate increased histamine synthesis in spleen and bone marrow cultures and in vivo.

Biological activity of hIL-3 and hIL-3 mutant proteins of the present invention is determined by DNA synthesis by human acute myelogenous leukemia cells (AML). The factor-dependent cell line AML 193 was adapted for use in testing biological activity.

One object of the present invention is to provide hIL-3 muteins and hIL-3 deletion muteins with four or more amino acid substitutions in the polypeptide sequence which have similar or improved biological activity in relation to native hIL-3 or native (15-125)hIL-3.

The present invention includes mutant polypeptides comprising minimally amino acids residues 15 to 118 of hIL-3 with or without additional amino acid extensions to the N-terminus and/or C-terminus which further contain four or more amino acid substitutions in the amino acid sequence of the polypeptide. It has been found that the (15-125)hIL-3 mutant is more soluble than is hIL-3 when expressed in the cytoplasm of *E. coli*, and the protein is secreted to the periplasm in *E. coli* at higher levels compared to native hIL-3.

When expressed in the *E. coli* cytoplasm, the above-mentioned mutant hIL-3 polypeptides of the present invention may also be constructed with Met-Ala- at the N-terminus so that upon expression the Met is cleaved off leaving Ala at the N-terminus. These mutant hIL-3 polypeptides may also be expressed in *E. coli* by fusing a signal peptide to the N-terminus. This signal peptide is cleaved from the polypeptide as part of the secretion process. Secretion in *E. coli* can be used to obtain the correct amino acid at the N-terminus (e.g., Asn<sup>15</sup> in the

(15-125) hIL-3 polypeptide) due to the precise nature of the signal peptidase. This is in contrast to the heterogeneity often observed at the N-terminus of proteins expressed in the cytoplasm in *E. coli*.

5       The hIL-3 mutant polypeptides of the present invention may have hIL-3 or hIL-3-like activity. For example, they may possess one or more of the biological activities of native hIL-3 and may be useful in stimulating the production of hematopoietic cells by human or primate  
10 progenitor cells. The hIL-3 muteins of the present invention and pharmaceutical compositions containing them may be useful in the treatment of conditions in which hematopoietic cell populations have been reduced or destroyed due to disease or to treatments such as radiation  
15 or chemotherapy.

hIL-3 muteins of the present invention may also be useful as antagonists which block the hIL-3 receptor by binding specifically to it and preventing binding of the agonist.

20       One potential advantage of the (15-125) hIL-3 muteins of the present invention, particularly those which retain activity similar to or better than that of native hIL-3, is that it may be possible to use a smaller amount of the biologically active mutein to produce the desired  
25 therapeutic effect. This may make it possible to reduce the number of treatments necessary to produce the desired therapeutic effect. The use of smaller amounts may also reduce the possibility of any potential antigenic effects or other possible undesirable side effects. For example,  
30 if a desired therapeutic effect can be achieved with a smaller amount of polypeptide it may be possible to reduce or eliminate side effects associated with the administration of native IL-3 such as the stimulation of leukotriene and/or histamine release. The hIL-3 muteins of  
35 the present invention may also be useful in the activation of stem cells or progenitors which have low receptor numbers. Pharmaceutical compositions containing (15-125) hIL-3 muteins of the present invention can be administered



parenterally, intravenously, or subcutaneously.

As another aspect of the present invention, there is provided a novel method for producing the novel family of human IL-3 muteins. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a vector containing a DNA sequence coding for expression of a novel hIL-3 mutant polypeptide. Suitable cells or cell lines may be bacterial cells. For example, the various strains of *E. coli* are well-known as host cells in the field of biotechnology. Examples of such strains include *E. coli* strains JM101 [Yanish-Perron, et al. (1985)] and MON105 [Obukowicz, et al. (1992)]. Various strains of *E. subtilis* may also be employed in this method. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention.

Also suitable for use in the present invention are mammalian cells, such as Chinese hamster ovary cells (CHO). General methods for expression of foreign genes in mammalian cells are reviewed in: Kaufman, R. J. (1987) High level production of proteins in mammalian cells, in Genetic Engineering, Principles and Methods, Vol. 9, J. K. Setlow, editor, Plenum Press, New York. An expression vector is constructed in which a strong promoter capable of functioning in mammalian cells drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant. For example, plasmids such as pcDNA I/Neo, pRc/RSV, and pRc/CMV (obtained from Invitrogen Corp., San Diego, California) can be used. The eukaryotic secretion signal peptide coding region can be from the hIL-3 gene itself or it can be from another secreted mammalian protein (Bayne, M. L. et al. (1987) Proc. Natl. Acad. Sci. USA 84, 2638-2642). After construction of the vector containing the hIL-3 variant gene, the vector DNA is transfected into mammalian cells. Such cells can be, for example, the COS7, HeLa, BHK, CHO, or mouse L lines. The cells can be cultured, for example, in DMEM media (JRH Scientific). The

hIL-3 variant secreted into the media can be recovered by standard biochemical approaches following transient expression 24 - 72 hours after transfection of the cells or after establishment of stable cell lines following selection for neomycin resistance. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al., U.S. Pat. No. 4,419,446. Another suitable mammalian cell line is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein. In addition, general methods for expression of foreign genes in insect cells using Baculovirus vectors are described in: Summers, M. D. and Smith, G. E. (1987) - A manual of methods for Baculovirus vectors and insect cell culture procedures, Texas Agricultural Experiment Station Bulletin No. 1555. An expression vector is constructed comprising a Baculovirus transfer vector, in which a strong Baculovirus promoter (such as the polyhedron promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant polypeptide. For example, the plasmid pVL1392 (obtained from Invitrogen Corp., San Diego, California) can be used. After construction of the vector carrying the hIL-3 variant gene, two micrograms of this DNA is cotransfected with one microgram of Baculovirus DNA (see Summers & Smith, 1987) into insect cells, strain SF9. Pure recombinant Baculovirus carrying the hIL-3 variant is used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas). The hIL-3 variant secreted into the medium can be recovered by

standard biochemical approaches.

Another aspect of the present invention provides plasmid DNA vectors for use in the method of expression of these novel hIL-3 muteins. These vectors contain the novel DNA sequences described above which code for the novel polypeptides of the invention. Appropriate vectors which can transform microorganisms capable of expressing the hIL-3 muteins include expression vectors comprising nucleotide sequences coding for the hIL-3 muteins joined to transcriptional and translational regulatory sequences which are selected according to the host cells used.

Vectors incorporating modified sequences as described above are included in the present invention and are useful in the production of the hIL-3 mutant polypeptides. The vector employed in the method also contains selected regulatory sequences in operative association with the DNA coding sequences of the invention and capable of directing the replication and expression thereof in selected host cells.

Additional details may be found in co-filed United States Patent Application Attorney docket number 2713/1, which is hereby incorporated by reference in its entirety.

All references, patents or applications cited herein are incorporated by reference in their entirety.

The present invention also includes the construction and expression of (15-125)human interleukin-3 muteins having four or more amino acid substitutions in secretion vectors that optimize accumulation of correctly folded, active polypeptide. While many heterologous proteins have been secreted in *E. coli* there is still a great deal of unpredictability and limited success (Stader and Silhavy 1990). Full-length hIL-3 is such a protein, where attempts to secrete the protein in *E. coli* resulted in low secretion levels. Secretion of the variant (15-125) hIL-3 mutant polypeptides of the present invention as a fusion with a signal peptide such as LamB results in correctly folded

protein that can be removed from the periplasm of E. coli by osmotic shock fractionation. This property of the variant (15-125) hIL-3 muteins allows for the direct and rapid screening for bioactivity of the secreted material in the crude osmotic shock fraction, which is a significant advantage. Furthermore, it provides a means of using the (15-125)hIL-3 muteins to conduct structure activity relationship (SAR) studies of the hIL-3 molecule. A further advantage of secretion of (15-125) hIL-3 muteins fused to the LamB signal peptide is that the secreted polypeptide has the correct N-terminal amino acid (Asn) due to the precise nature of the cleavage of the signal peptide by signal peptidase, as part of the secretion process.

The (15-125)hIL-3 muteins of the present invention may include hIL-3 polypeptides having Met-, Ala- or Met-Ala- attached to the N-terminus. When the muteins are expressed in the cytoplasm of E. coli, polypeptides with and without Met attached to the N-terminus are obtained. The methionine can in some cases be removed by methionine aminopeptidase.

Amino terminal sequences of hIL-3 muteins made in E. coli were determined using the method described by Hunkapillar et al., (1983). It was found that hIL-3 proteins made in E. coli from genes encoding Met-(15-125)hIL-3 were isolated as Met-(15-125) hIL-3. Proteins produced from genes encoding Met-Ala-(15-125) hIL-3 were produced as Ala-(15-125) hIL-3. The N-termini of proteins made in the cytoplasm of E. coli are affected by posttranslational processing by methionine aminopeptidase (Ben-Bassat et al., 1987) and possibly by other peptidases.

One method of creating the preferred hIL-3 (15-125) mutant genes is cassette mutagenesis [Wells, et al. (1985)] in which a portion of the coding sequence of hIL-3 in a plasmid is replaced with synthetic oligonucleotides that encode the desired amino acid substitutions in a portion of the gene between two restriction sites. In a similar manner amino acid substitutions could be made in the full-length hIL-3 gene, or genes encoding variants of hIL-3 in

which from 1 to 14 amino acids have been deleted from the N-terminus and/or from 1 to 15 amino acids have been deleted from the C-terminus. When properly assembled these oligonucleotides would encode hIL-3 variants with the  
5 desired amino acid substitutions and/or deletions from the N-terminus and/or C-terminus. These and other mutations could be created by those skilled in the art by other mutagenesis methods including; oligonucleotide-directed mutagenesis [Zoller and Smith (1982, 1983, 1984), Smith  
10 (1985), Kunkel (1985), Taylor, et al. (1985), Deng and Nickoloff (1992)] or polymerase chain reaction (PCR) techniques [Saiki, (1985)].

Pairs of complementary synthetic oligonucleotides encoding portions of the amino terminus of the hIL-3 gene  
15 can be made and annealed to each other. Such pairs would have protruding ends compatible with ligation to NcoI at one end. The NcoI site would include the codon for the initiator methionine. At the other end of oligonucleotide pairs, the protruding (or blunt) ends would be compatible  
20 with a restriction site that occurs within the coding sequence of the hIL-3 gene. The DNA sequence of the oligonucleotide would encode sequence for amino acids of hIL-3 with the exception of those substituted and/or deleted from the sequence.

25 The NcoI enzyme and the other restriction enzymes chosen should have recognition sites that occur only once in the DNA of the plasmid chosen. Plasmid DNA can be treated with the chosen restriction endonucleases then ligated to the annealed oligonucleotides. The ligated  
30 mixtures can be used to transform competent JM101 cells to resistance to an appropriate antibiotic. Single colonies can be picked and the plasmid DNA examined by restriction analysis and/or DNA sequencing to identify plasmids with mutant hIL-3 genes.

35 One example of a restriction enzyme which cleaves within the coding sequence of the hIL-3 gene is ClaI whose recognition site is at codons 20 and 21. The use of ClaI to cleave the sequence of hIL-3 requires that the plasmid

DNA be isolated from an E. coli strain that fails to methylate adenines in the DNA at GATC recognition sites. This is because the recognition site for ClaI, ATCGAT, occurs within the sequence GATCGAT which occurs at codons 19, 20 and 21 in the hIL-3 gene. The A in the GATC sequence is methylated in most E. coli host cells. This methylation prevents ClaI from cleaving at that particular sequence. An example of a strain that does not methylate adenines is GM48.

10 Interpretation of activity of single amino acid mutants in IL-3 (15-125)

As illustrated in Tables 6 and 9, there are certain positions in the IL-3 (15-125) molecule which are intolerant of substitutions, in that most or all substitutions at these positions resulted in a considerable decrease in bioactivity. There are two likely classes of such "down-mutations": mutations that affect overall protein structure, and mutations that interfere directly with the interaction between the IL-3 molecule and its receptor. Mutations affecting the three-dimensional structure of the protein will generally lie in the interior of the protein, while mutations affecting receptor binding will generally lie on the surface of the protein. Although the three-dimensional structure of IL-3 is unknown, there are simple algorithms which can aid in the prediction of the structure. One such algorithm is the use of "helical wheels" (Kaiser, E.T. & Kezdy, F.J., Science, 223:249-255 (1984)). In this method, the presence of alpha helical protein structures can be predicted by virtue of their amphipathic nature. Helices in globular proteins commonly have an exposed hydrophilic side and a buried hydrophobic side. As a broad generalization, in globular proteins, hydrophobic residues are present in the interior of the protein, and hydrophilic residues are present on the surface. By displaying the amino acid sequence of a protein on such a "helical wheel" it is possible to derive a model for which amino acids in alpha helices are exposed

and which are buried in the core of the protein. Such an analysis of the IL-3 (15-125) molecule predicts that the following helical residues are buried in the core:

5           M19, I20, I23, I24, L27, L58, F61, A64, L68, A71, I74,  
I77, L78, L81, W104, F107, L111, Y114, L115, L118.

10           In addition, cysteine residues at positions 16 and 84  
are linked by a disulfide bond, which is important for the  
overall structure or "folding" of the protein. Finally,  
15           mutations which result in a major disruption of the protein  
structure may be expressed at low level in the secretion  
system used in our study, for a variety of reasons: either  
because the mis-folded protein is poorly recognized by the  
15           secretion machinery of the cell; because mis-folding of the  
protein results in aggregation, and hence the protein  
cannot be readily extracted from the cells; or because the  
mis-folded protein is more susceptible to degradation by  
cellular proteases. Hence, a block in secretion may  
20           indicate which positions in the IL-3 molecule which are  
important for maintenance of correct protein structure.

25           In order to retain the activity of a variant of IL-3,  
it is necessary to retain both the structural integrity of  
the protein, and retain the specific residues important for  
receptor contact. Hence it is possible to define specific  
amino acid residues in IL-3 (15-125) which must be retained  
in order to preserve biological activity.

30           Residues predicted to be important for interaction  
with the receptor: D21, E22, E43, D44, L48, R54, R94,  
D103, K110, F113.

35           Residues predicted to be structurally important: C16,  
L58, F61, A64, I74, L78, L81, C84, P86, P92, P96, F107,  
L111, L115, L118.

The hIL-3 muteins of the present invention may be useful in the treatment of diseases characterized by a decreased levels of either myeloid, erythroid, lymphoid, or megakaryocyte cells of the hematopoietic system or combinations thereof. In addition, they may be used to activate mature myeloid and/or lymphoid cells. Among conditions susceptible to treatment with the polypeptides of the present invention is leukopenia, a reduction in the number of circulating leukocytes (white cells) in the peripheral blood. Leukopenia may be induced by exposure to certain viruses or to radiation. It is often a side effect of various forms of cancer therapy, e.g., exposure to chemotherapeutic drugs and of infection or hemorrhage. Therapeutic treatment of leukopenia with these hIL-3 mutant polypeptides of the present invention may avoid undesirable side effects caused by treatment with presently available drugs.

The hIL-3 muteins of the present invention may be useful in the treatment of neutropenia and, for example, in the treatment of such conditions as aplastic anemia, cyclic neutropenia, idiopathic neutropenia, Chediak-Higashi syndrome, systemic lupus erythematosus (SLE), leukemia, myelodysplastic syndrome and myelofibrosis.

Many drugs may cause bone marrow suppression or hematopoietic deficiencies. Examples of such drugs are AZT, DDI, alkylating agents and anti-metabolites used in chemotherapy, antibiotics such as chloramphenicol, penicillin and sulfa drugs, phenothiazones, tranquilizers such as meprobamate, and diuretics. The hIL-3 muteins of the present invention may be useful in preventing or treating the bone marrow suppression or hematopoietic deficiencies which often occur in patients treated with these drugs.

Hematopoietic deficiencies may also occur as a result of viral, microbial or parasitic infections and as a result of treatment for renal disease or renal failure, e.g., dialysis. The hIL-3 muteins of the present invention may be useful in treating such hematopoietic deficiency.



The treatment of hematopoietic deficiency may include administration of the hIL-3 mutein of a pharmaceutical composition containing the hIL-3 mutein to a patient. The hIL-3 muteins of the present invention may also be useful for the activation and amplification of hematopoietic precursor cells by treating these cells in vitro with the muteins of the present invention prior to injecting the cells into a patient.

Various immunodeficiencies e.g., in T and/or B lymphocytes, or immune disorders, e.g., rheumatoid arthritis, may also be beneficially affected by treatment with the hIL-3 mutant polypeptides of the present invention. Immunodeficiencies may be the result of viral infections e.g. HTLVI, HTLVII, HTLVIII, severe exposure to radiation, cancer therapy or the result of other medical treatment. The hIL-3 mutant polypeptides of the present invention may also be employed, alone or in combination with other hematopoietins, in the treatment of other blood cell deficiencies, including thrombocytopenia (platelet deficiency), or anemia. Other uses for these novel polypeptides are in the treatment of patients recovering from bone marrow transplants in vivo and ex vivo, and in the development of monoclonal and polyclonal antibodies generated by standard methods for diagnostic or therapeutic use.

Other aspects of the present invention are methods and therapeutic compositions for treating the conditions referred to above. Such compositions comprise a therapeutically effective amount of one or more of the hIL-3 muteins of the present invention in a mixture with a pharmaceutically acceptable carrier. This composition can be administered either parenterally, intravenously or subcutaneously. When administered, the therapeutic composition for use in this invention is preferably in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity, stability and the like, is within the skill of

the art.

The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician considering various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, a daily regimen may be in the range of 0.2 - 150  $\mu\text{g/kg}$  of non-glycosylated IL-3 protein per kilogram of body weight. This dosage regimen is referenced to a standard level of biological activity which recognizes that native IL-3 generally possesses an  $\text{EC}_{50}$  at or about 10 picoMolar to 100 picoMolar in the AML proliferation assay described herein. Therefore, dosages would be adjusted relative to the activity of a given mutein vs. the activity of native (reference) IL-3 and it would not be unreasonable to note that dosage regimens may include doses as low as 0.1 microgram and as high as 1 milligram per kilogram of body weight per day. In addition, there may exist specific circumstances where dosages of IL-3 mutein would be adjusted higher or lower than the range of 10 - 200 micrograms per kilogram of body weight. These include co-administration with other CSF or growth factors; co-administration with chemotherapeutic drugs and/or radiation; the use of glycosylated IL-3 mutein; and various patient-related issues mentioned earlier in this section. As indicated above, the therapeutic method and compositions may also include co-administration with other human factors. A non-exclusive list of other appropriate hematopoietins, CSFs and interleukins for simultaneous or serial co-administration with the polypeptides of the present invention includes GM-CSF, CSF-1, G-CSF, Meg-CSF, M-CSF, erythropoietin (EPO), IL-1, IL-4, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, LIF, B-cell growth factor, B-cell differentiation factor and eosinophil differentiation factor, stem cell factor (SCF) also known as steel factor or c-kit ligand, or combinations thereof. The dosage recited above would be adjusted to compensate

for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by periodic assessment of the hematological profile, e.g., differential cell count and the like.

5 Materials and methods for hIL-3 Mutein Expression in E. coli

Unless noted otherwise, all specialty chemicals were obtained from Sigma Co., (St. Louis, MO). Restriction endonucleases, T4 poly-nucleotides kinase, E. coli DNA polymerase I large fragment (Klenow) and T4 DNA ligase were obtained from New England Biolabs (Beverly, Massachusetts).  
10 Escherichia coli strains

Strain JM101: delta (pro lac), supE, thi, F'(traD36, rpoAB, lacI-Q, lacZdeltaM15) (Messing, 1979). This strain can be obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, accession number 33876. MON 105 (W3110 rpoH358) is a derivative of W3110 (Bachmann, 1972) and has been assigned ATCC accession number 55204. Strain GM48: dam-3, dcm-6, gal, ara, lac, thr, leu, tonA, tsx (Marinus, 1973) was used to make plasmid DNA that is not methylated at the sequence GATC.  
20

Genes and plasmids

The gene used for hIL-3 production in E. coli was obtained from British Biotechnology Incorporated, Cambridge, England, catalogue number BBG14. This gene is carried on a pUC based plasmid designated pP0518.  
25

The plasmids used for production of hIL-3 in E. coli contain genetic elements whose use has been described (Olins et al., 1988; Olins and Rangwala, 1990). The replicon used is that of pBR327 (Covarrubias, et al., 1981) which is maintained at a copy number of about 100 in the cell (Soberon et al., 1980). A gene encoding the beta-lactamase protein is present on the plasmids. This protein confers ampicillin resistance on the cell. This resistance serves as a selectable phenotype for the presence of the plasmid in the cell.  
30  
35

For cytoplasmic expression vectors the transcription

promoter was derived from the *recA* gene of *E. coli* (Sancar et al., 1980). This promoter, designated *precA*, includes the RNA polymerase binding site and the *lexA* repressor binding site (the operator). This segment of DNA provides  
5 high level transcription that is regulated even when the *recA* promoter is on a plasmid with the pBR327 origin of replication (Olins et al., 1988) incorporated herein by reference.

In secretion expression plasmids the transcription  
10 promoter was derived from the *ara B*, *A*, and *D* genes of *E. coli* (Greenfield et al., 1978). This promoter is designated *pAraBAD* and is contained on a 323 base pair *SacII*, *BglIII* restriction fragment. The *LamB* secretion leader (Wong et al., 1988, Clement et al., 1981) was fused  
15 to the N-terminus of the *hIL-3* gene at the recognition sequence for the enzyme *NcoI* (5'CCATGG3'). The *hIL-3* genes used were engineered to have a *HindIII* recognition site (5'AAGCTT3') following the coding sequence of the gene.

These *hIL-3* variants were expressed as a fusion with  
20 the *LamB* signal peptide shown in Figure 8, operatively joined to the *araBAD* promoter (Greenfield, 1978) and the *gl0-L* ribosome binding site (Olins et al. 1988). The processed form was selectively released from the periplasm by osmotic shock as a correctly folded and fully active  
25 molecule. Secretion of (15-125) *hIL-3* was further optimized by using low inducer (arabinose) concentration and by growth at 30°C. These conditions resulted in lower accumulation levels of unprocessed *LamB* signal peptide (15-125) *hIL-3* fusion, maximal accumulation levels of processed  
30 (15-125) *hIL-3* and selective release of (15-125) *hIL-3* by osmotic shock fractionation. The use of a tightly regulated promoter such as *araBAD* from which the transcription level and hence the expression level can be modulated allowed for the optimization of secretion of (15-  
35 125) *hIL-3*.

The ribosome binding site used is that from gene 10 of phage T7 (Olins et al., 1988). This is encoded in a 100 base pair (bp) fragment placed adjacent to *precA*. In the

plasmids used herein, the recognition sequence for the enzyme NcoI (CCATGG) follows the gl0-L. It is at this NcoI site that the hIL-3 genes are joined to the plasmid. It is expected that the nucleotide sequence at this junction will be recognized in mRNA as a functional start site for translation (Olins et al., 1988). The hIL-3 genes used were engineered to have a HindIII recognition site (AAGCTT) downstream from the coding sequence of the gene. At this HindIII site is a 514 base pair RsaI fragment containing the origin of replication of the single stranded phage f1 (Dente et al., 1983; Olins, et al., 1990) both incorporated herein by reference. A plasmid containing these elements is pMON2341. Another plasmid containing these elements is pMON5847 which has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 under the accession number ATCC 68912.

#### Synthesis of Oligonucleotides

Oligonucleotides were synthesized on Nucleotide Synthesizer model 380A or 380B from Applied Biosystems, Inc. (Foster City, California). Oligonucleotides were purified by polyacrylamide gel electrophoresis at concentrations from 12 - 20% (19:1 crosslinked) in 0.5 x Tris borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA) followed by passage through a Nensorb column obtained from New England Nuclear (Boston, Massachusetts) using a PREP Automated Sample Processor obtained from DuPont, Co. (Wilmington, Delaware).

#### Quantitation of synthetic oligonucleotides

Synthetic oligonucleotides were resuspended in water and quantitated by reading the absorbance at 260nm on a Beckman DU40 Spectrophotometer (Irvine, California) using a one centimeter by one millimeter quartz cuvette (Maniatis, 1982). The concentration was determined using an extinction coefficient of  $1 \times 10^4$  (Voet et al., 1963; Mahler and Cordes, 1966). The oligonucleotides were then diluted to a desired concentration.

Quantitation of synthetic DNA fragments can also be achieved by adding 10 to 100 picomoles of DNA to a solution

containing kinase buffer (25 mM Tris pH 8.0, 10 mM MgCl<sub>2</sub>, 10 mM DTT and 2 mM spermidine). To the reaction mix is added ATP to 20 micromolar, ATP radiolabeled at the gamma phosphate (5000-10,000 dpm/pmol) and 5 units of T4 polynucleotide kinase. Radiolabelled material is obtained from New England Nuclear (Boston, Massachusetts). The 10 microliter mixture is incubated at 37°C for one hour. A 1 microliter aliquot of the mixture was chromatographed on DEAE paper (Whatman) in 0.3 M ammonium bicarbonate. The counts that remained at the origin were used to determine the concentration of the synthetic DNA.

#### Recombinant DNA methods

Isolation of plasmid DNA from *E. coli* cultures was performed as described (Birnboim and Doly, 1979). Some DNAs were purified by Magic™ columns, available from Promega (Madison, Wisconsin).

Purified plasmid DNA was treated with restriction endonucleases according to manufacturer's instructions. Analysis of the DNA fragments produced by treatment with restriction enzymes was done by agarose or polyacrylamide gel electrophoresis. Agarose (DNA grade from Fisher, Pittsburgh PA.) was used at a concentration of 1.0% in a Tris-acetate running buffer (0.04 M Tris-acetate, 0.001M EDTA). Polyacrylamide (BioRad, Richmond CA.) was used at a concentration of 6% (19:1 crosslinked) in 0.5 X Tris-borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA), hereafter referred to as PAGE.

DNA polymerase I, large fragment, Klenow enzyme was used according to manufacturers instructions to catalyze the addition of mononucleotides from 5' to 3' of DNA fragments which had been treated with restriction enzymes that leave protruding ends. The reactions were incubated at 65°C for 10 minutes to heat inactivate the Klenow enzyme.

The synthetic oligonucleotides were made without 5' or 3' terminal phosphates. In cases where such oligonucleotides were ligated end to end, the oligonucleotides were treated at a concentration of

10 picomoles per microliter with T4 polynucleotide kinase in the following buffer: 25 mM Tris, pH 8.0, 10 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 2 mM spermidine, 1 mM rATP. After incubation for 30 minutes at 37°C, the samples were  
5 incubated at 65°C for five minutes to heat inactivate the kinase.

#### Synthetic gene assembly

The (15-125) hIL-3 gene was divided into four regions separated by five convenient restriction sites. In each of  
10 the four regions synthetic oligonucleotides were designed so that they would anneal in complementary pairs, with protruding single stranded ends, and when the pairs were properly assembled would result in a DNA sequence that encoded a portion of the hIL-3 gene. Amino acid  
15 substitutions in the hIL-3 gene were made by designing the oligonucleotides to encode the desired substitutions. The complementary oligonucleotides were annealed at concentration of 1 picomole per microliter in ligation buffer plus 50mM NaCl. The samples were heated in a 100 ml  
20 beaker of boiling water and permitted to cool slowly to room temperature. One picomole of each of the annealed pairs of oligonucleotides were ligated with approximately 0.2 picomoles of plasmid DNA, digested with the appropriate restriction enzymes, in ligation buffer (25 mM Tris pH 8.0,  
25 10 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 1 mM ATP, 2mM spermidine) with T4 DNA ligase obtained from New England Biolabs (Beverly, Massachusetts) in a total volume of 20 µl at room temperature overnight.

DNA fragments were isolated from agarose gels by  
30 intercepting the restriction fragments on DEAE membranes from Schleicher and Schuell (Keene, New Hampshire) and eluting the DNA in 10 mM Tris, 1 mM EDTA, 1 M NaCl at 55°C for 1 hour, according to manufacturer's directions. The solutions containing the DNA fragment were concentrated and  
35 desalted by using Centricon 30 concentrators from Amicon (W.R. Grace, Beverly MA.) according to the manufacturer's directions. Ligations were performed at 15°C overnight, except as noted, in ligation buffer.

### Polymerase Chain Reaction

Polymerase Chain Reaction (hereafter referred to as PCR) techniques (Saiki, 1985) used the reagent kit and thermal cycler from Perkin-Elmer Cetus (Norwalk, CT.). PCR is based on a thermostable DNA polymerase from *Thermus aquaticus*. The PCR technique is a DNA amplification method that mimics the natural DNA replication process in that the number of DNA molecules doubles after each cycle, in a way similar to in vivo replication. The DNA polymerase mediated extension is in a 5' to 3' direction. The term "primer" as used herein refers to an oligonucleotide sequence that provides an end to which the DNA polymerase can add nucleotides that are complementary to a nucleotide sequence. The latter nucleotide sequence is referred to as the "template", to which the primers are annealed. The amplified PCR product is defined as the region comprised between the 5' ends of the extension primers. Since the primers have defined sequences, the product will have discrete ends, corresponding to the primer sequences. The primer extension reaction was carried out using 20 picomoles (pmoles) of each of the oligonucleotides and 1 picogram of template plasmid DNA for 35 cycles (1 cycle is defined as 94 degrees C for one minute, 50 degrees C for two minutes and 72 degrees for three minutes.). The reaction mixture was extracted with an equal volume of phenol/chloroform (50% phenol and 50% chloroform, volume to volume) to remove proteins. The aqueous phase, containing the amplified DNA, and solvent phase were separated by centrifugation for 5 minutes in a microcentrifuge (Model 5414 Eppendorf Inc, Fremont CA.). To precipitate the amplified DNA the aqueous phase was removed and transferred to a fresh tube to which was added 1/10 volume of 3M NaOAc (pH 5.2) and 2.5 volumes of ethanol (100% stored at minus 20 degrees C). The solution was mixed and placed on dry ice for 20 minutes. The DNA was pelleted by centrifugation for 10 minutes in a microcentrifuge and the solution was removed from the pellet. The DNA pellet was washed with 70%



ethanol, ethanol removed and dried in a speedvac concentrator (Savant, Farmingdale, New York). The pellet was resuspended in 25 microliters of TE (20mM Tris-HCl pH 7.9, 1mM EDTA). Alternatively the DNA was precipitated by adding equal volume of 4M NH<sub>4</sub>OAc and one volume of isopropanol [Treco et al., (1988)]. The solution was mixed and incubated at room temperature for 10 minutes and centrifuged. These conditions selectively precipitate DNA fragments larger than ~ 20 bases and were used to remove oligonucleotide primers. One quarter of the reaction was digested with restriction enzymes [Higuchi, (1989)] and on completion heated to 70 degrees C to inactivate the enzymes.

15 Recovery of recombinant plasmids from ligation mixes

E. coli JM101 cells were made competent to take up DNA. Typically, 20 to 100 ml of cells were grown in LB medium to a density of approximately 150 Klett units and then collected by centrifugation. The cells were resuspended in one half culture volume of 50 mM CaCl<sub>2</sub> and held at 4°C for one hour. The cells were again collected by centrifugation and resuspended in one tenth culture volume of 50 mM CaCl<sub>2</sub>. DNA was added to a 150 microliter volume of these cells, and the samples were held at 4°C for 30 minutes. The samples were shifted to 42°C for one minute, one milliliter of LB was added, and the samples were shaken at 37°C for one hour. Cells from these samples were spread on plates containing ampicillin to select for transformants. The plates were incubated overnight at 37°C. Single colonies were picked, grown in LB supplemented with ampicillin overnight at 37°C with shaking. From these cultures DNA was isolated for restriction analysis.

35 Culture medium

LB medium (Maniatis et al., 1982) was used for growth of cells for DNA isolation. M9 minimal medium supplemented with 1.0% casamino acids, acid hydrolyzed casein, Difco

(Detroit, Michigan) was used for cultures in which recombinant hIL-3 was produced. The ingredients in the M9 medium were as follows: 3g/liter  $\text{KH}_2\text{PO}_4$ , 6g/l  $\text{Na}_2\text{HPO}_4$ , 0.5 g/l  $\text{NaCl}$ , 1 g/l  $\text{NH}_4\text{Cl}$ , 1.2 mM  $\text{MgSO}_4$ , 0.025 mM  $\text{CaCl}_2$ , 0.2% glucose (0.2% glycerol with the AraBAD promoter), 1% casamino acids, 0.1 ml/l trace minerals (per liter 108 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 4.0 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 7.0  $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$ , 7.0 g  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 8.0 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 2.0 g  $\text{H}_3\text{BO}_3$ , 5.0 g  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 100 ml concentrated  $\text{HCl}$ ). Bacto agar was used for solid media and ampicillin was added to both liquid and solid LB media at 200 micrograms per milliliter.

#### DNA sequence analysis

The nucleotide sequencing of plasmid DNA was determined using a Genesis 2000 sequencer obtained from DuPont (Wilmington, Delaware) according to the methods of Prober et al. (1987) and Sanger et al. (1977). Some DNA sequences were performed using Sequenase<sup>TM</sup> polymerase (U.S. Biochemicals, Cleveland, Ohio) according to manufacturer's directions.

#### Production of recombinant hIL-3 muteins in E. coli with vectors employing the recA promoter

E. coli strains harboring the plasmids of interest were grown at 37°C in M9 plus casamino acids medium with shaking in a Gyrotory water bath Model G76 from New Brunswick Scientific (Edison, New Jersey). Growth was monitored with a Klett Summerson meter (green 54 filter), Klett Mfg. Co. (New York, New York). At a Klett value of approximately 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining culture, nalidixic acid (10mg/ml) in 0.1 N NaOH was added to a final concentration of 50 µg/ml. The cultures were shaken at 37°C for three to four hours after addition of nalidixic acid. A high degree of aeration was maintained throughout the bacterial growth in order to achieve maximal production of the desired gene product. The cells were examined under a light microscope for the

presence of refractile bodies (RBs). One milliliter aliquots of the culture were removed for analysis of protein content.

Production of recombinant hIL-3 proteins from the pAraBAD promoter in *E. coli*

5 *E. coli* strains harboring the plasmids of interest were grown at 30°C with shaking in M9 medium plus casamino acids and glycerol. Growth was monitored with a Klett Summerson colorimeter, using a green 54 filter. At a Klett  
10 value of about 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining culture, 20% arabinose was added to a final concentration of 0.05%. The cultures were shaken at 30°C  
15 for three to four hours after addition of arabinose. A high degree of aeration was maintained throughout the bacterial growth in order to achieve maximal production of the desired gene product. One milliliter aliquots of the culture were removed for analysis of protein content.

Secretion and osmotic shock

20 Three hour post induction samples were fractionated by osmotic shock [Neu and Heppel (1965)]. The optical density (Klett value) of the cultures was determined and 1 ml of cells were centrifuged in a Sigma microcentrifuge (West Germany) model 202MK in 1.5 mls snap top microcentrifuge  
25 tubes for 5 minutes at 10,000 rpm. The cell pellet was resuspended very gently by pipeting in a room temperature sucrose solution (20% sucrose w/v, 30mM Tris-HCl pH7.5, 1mM EDTA), using 1µl/1 Klett unit. Following a 10 minute incubation at room temperature, the cells were centrifuged  
30 for 5 minutes at 10,000 rpm. The sucrose fraction was carefully removed from the cell pellet. The cell pellet was then resuspended very gently by pipeting in ice cold distilled water, using 1µl/1 Klett unit. Following a 10 minute incubation on ice, the cells were centrifuged for 5  
35 minutes at 12,000 rpm. The water fraction was carefully removed. Equal volumes of the sucrose and water fractions were pooled and aliquoted to provide samples for activity screening.

Analysis of protein content of E. coli cultures producing hIL-3 mutant polypeptides

- Bacterial cells from cultures treated as described above were collected from the medium by centrifugation.
- 5 Aliquots of these cells were resuspended in SDS loading buffer (4X: 6 g SDS, 10 ml beta-mercaptoethanol, 25 ml upper Tris gel stock (0.5 M Tris HCl pH 6.8, 0.4% SDS) brought to 50 ml with glycerol, 0.2% bromophenol blue was added) at a concentration of one microliter per Klett unit.
- 10 These samples were incubated at 85°C for five minutes and vortexed. Five or ten microliter aliquots of these samples were loaded on 15% polyacrylamide gels prepared according to the method of Laemmli (1970). Protein bands were visualized by staining the gels with a solution of acetic acid, methanol and water at 5:1:5 ratio (volume to volume)
- 15 to which Coomassie blue had been added to a final concentration of 1%. After staining, the gels were washed in the same solution without the Coomassie blue and then washed with a solution of 7% acetic acid, 5% methanol.
- 20 Gels were dried on a gel drier Model SE1160 obtained from Hoeffler (San Francisco, California). The amount of stained protein was measured using a densitometer obtained from Joyce-Loebl (Gateshead, England). The values obtained were a measure of the amount of the stained hIL-3 protein
- 25 compared to the total of the stained protein of the bacterial cells.

Western blot analysis of hIL-3 mutants made in E. coli

- In some E. coli cultures producing hIL-3, the level of accumulation of the hIL-3 protein is lower than 5% of total
- 30 bacterial protein. To detect hIL-3 produced at this level, Western blot analysis was used. Proteins from cultures induced with nalidixic acid or arabinose were run on polyacrylamide gels as described above except that volumes of sample loaded were adjusted to produce appropriate
- 35 signals. After electrophoresis, the proteins were electroblotted to APT paper, Transa-bind, Schleicher and Schuell (Keene, New Hampshire) according to the method of Renart et al. (1979). Antisera used to probe these blots

had been raised in rabbits, using peptides of the sequence of amino acids 20 to 41 and 94 to 118 of hIL-3 as the immunogens. The presence of bound antibody was detected with Staphylococcal protein A radiolabeled with  $^{125}\text{I}$ ,  
5 obtained from New England Nuclear (Boston, Massachusetts).  
Fractionation of E. coli cells producing hIL-3 proteins in the cytoplasm

Cells from E. coli cultures harboring plasmids that produce hIL-3 muteins were induced with nalidixic acid.  
10 After three hours, the hIL-3 muteins accumulated in refractile bodies. The first step in purification of the hIL-3 muteins was to sonicate cells. Aliquots of the culture were resuspended from cell pellets in sonication buffer: 10 mM Tris, pH 8.0, 1 mM EDTA, 50 mM NaCl and 0.1  
15 mM PMSF. These resuspended cells were subjected to several repeated sonication bursts using the microtip from a Sonicator cell disrupter, Model W-375 obtained from Heat Systems-Ultrasonics Inc. (Farmingdale, New York). The extent of sonication was monitored by examining the  
20 homogenates under a light microscope. When nearly all of the cells had been broken, the homogenates were fractionated by centrifugation. The pellets, which contain most of the refractile bodies, are highly enriched for hIL-3 muteins.

25 Methods: Extraction, Refolding and Purification of Interleukin-3 (IL-3) Muteins Expressed as Refractile Bodies in E. coli.

Extraction of refractile bodies (RB's):

For each gram of RB's (and typically one gram is  
30 obtained from a 300 ml E. coli culture), 5 ml of a solution containing 6M guanidine hydrochloride (GnHCl), 50 mM 2-N-cyclohexylaminoethanesulfonic acid (CHES) pH 9.5 and 20 mM dithiothreitol (DTT) was added. The RB's were extracted with a Bio-Homogenizer for 15-30 seconds and gently rocked  
35 for 2 hours at 5 degrees centigrade ( $5^{\circ}\text{C}$ ) to allow the protein to completely reduce and denature.

### Refolding of the IL-3 muteins

The protein solution was transferred to dialysis tubing (1000 molecular weight cut-off) and dialyzed against at least 100 volumes of 4M GnHCl - 50 mM CHES pH 8.0. The dialysis was continued overnight at 5°C while gently stirring. Subsequently dialysis was continued against at least 100 volumes of 2M GnHCl - 50 mM CHES pH 8.0 and dialyzed overnight at 5°C while gently stirring.

### Purification of the IL-3 muteins

The protein solution was removed from the dialysis tubing and acidified by the addition of 40% acetonitrile (CH<sub>3</sub>CN) - 0.2% trifluoroacetic acid (TFA) to a final concentration of 20% CH<sub>3</sub>CN - 0.1% TFA. This was centrifuged (16,000 x g for 5 minutes) to clarify and the supernatant was loaded onto a Vydac C-18 reversed phase column (10x250 mm) available from Vydac (Hesperia, California) previously equilibrated in 20% CH<sub>3</sub>CN - 0.1% TFA. The column was eluted with a linear gradient (0.2% CH<sub>3</sub>CN/minute) between 40 - 50% CH<sub>3</sub>CN - 0.1% TFA at a flow rate of 3 ml/minute while collecting 1.5 ml fractions. The fractions were analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) and the appropriate fractions pooled. The pooled material was dried by lyophilization or in a Speed Vac concentrator. The dry powder was reconstituted with 10 mM ammonium bicarbonate pH 7.5, centrifuged (16,000 x g for 5 minutes) to clarify and assayed for protein concentration by the method of Bradford (1976) with bovine serum albumin as the standard. Such protein can be further analyzed by additional techniques such as, SDS-PAGE, electrospray mass spectrometry, reverse phase HPLC, capillary zone electrophoresis, amino acid composition analysis, and ELISA (enzyme-linked immunosorbent assay).

### hIL-3 SANDWICH ELISA

IL-3 protein concentrations can be determined using a sandwich ELISA based on an affinity purified polyclonal goat anti-rhIL-3. Microtiter plates (Dynatech Immulon II)

were coated with 150  $\mu$ l goat-anti-rhIL-3 at a concentration of approximately 1  $\mu$ g/ml in 100 mM NaHCO<sub>3</sub>, pH 8.2. Plates were incubated overnight at room temperature in a chamber maintaining 100% humidity. Wells were emptied and the remaining reactive sites on the plate were blocked with 200  $\mu$ l of solution containing 10 mM PBS, 3% BSA and 0.05% Tween 20, pH 7.4 for 1 hour at 37° C and 100% humidity. Wells were emptied and washed 4X with 150 mM NaCl containing 0.05% Tween 20 (wash buffer). Each well then received 150  $\mu$ l of dilution buffer (10 mM PBS containing 0.1% BSA, 0.01% Tween 20, pH 7.4), containing rhIL-3 standard, control, sample or dilution buffer alone. A standard curve was prepared with concentrations ranging from 0.125 ng/ml to 5 ng/ml using a stock solution of rhIL-3 (concentration determined by amino acid composition analysis). Plates were incubated 2.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150  $\mu$ l of an optimal dilution (as determined in a checkerboard assay format) of goat anti-rhIL-3 conjugated to horseradish peroxidase. Plates were incubated 1.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150  $\mu$ l of ABTS substrate solution (Kirkegaard and Perry). Plates were incubated at room temperature until the color of the standard wells containing 5 ng/ml rhIL-3 had developed enough to yield an absorbance between 0.5-1.0 when read at a test wavelength of 410 nm and a reference wavelength of 570 nm on a Dynatech microtiter plate reader. Concentrations of immunoreactive rhIL-3 in unknown samples were calculated from the standard curve using software supplied with the plate reader.

#### AML Proliferation Assay for Bioactive Human Interleukin-3

The factor-dependent cell line AML 193 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This cell line, established from a patient with acute myelogenous leukemia, is a growth factor dependent cell

line which displayed enhanced growth in GM/CSF supplemented medium (Lange, B., et al., (1987); Valtieri, M., et al., (1987)). The ability of AML 193 cells to proliferate in the presence of human IL-3 has also been documented. (Santoli, D., et al., (1987)). A cell line variant was used, AML 193 1.3, which was adapted for long term growth in IL-3 by washing out the growth factors and starving the cytokine dependent AML 193 cells for growth factors for 24 hours. The cells were then replated at  $1 \times 10^5$  cells/well in a 24 well plate in media containing 100 U/ml IL-3. It took approximately 2 months for the cells to grow rapidly in IL-3. These cells were maintained as AML 193 1.3 thereafter by supplementing tissue culture medium (see below) with human IL-3.

AML 193 1.3 cells were washed 6 times in cold Hanks balanced salt solution (HBSS, Gibco, Grand Island, NY) by centrifuging cell suspensions at  $250 \times g$  for 10 minutes followed by decantation of supernatant. Pelleted cells were resuspended in HBSS and the procedure was repeated until six wash cycles were completed. Cells washed six times by this procedure were resuspended in tissue culture medium at a density ranging from  $2 \times 10^5$  to  $5 \times 10^5$  viable cells/ml. This medium was prepared by supplementing Iscove's modified Dulbecco's Medium (IMDM, Hazleton, Lenexa, KS) with albumin, transferrin, lipids and 2-mercaptoethanol. Bovine albumin (Boehringer-Mannheim, Indianapolis, IN) was added at 500  $\mu\text{g/ml}$ ; human transferrin (Boehringer-Mannheim, Indianapolis, IN) was added at 100  $\mu\text{g/ml}$ ; soybean lipid (Boehringer-Mannheim, Indianapolis, IN) was added at 50  $\mu\text{g/ml}$ ; and 2-mercaptoethanol (Sigma, St. Louis, MO) was added at  $5 \times 10^{-5}$  M.

Serial dilutions of human interleukin-3 or human interleukin-3 variant protein (hIL-3 mutein) were made in triplicate series in tissue culture medium supplemented as stated above in 96 well Costar 3596 tissue culture plates. Each well contained 50  $\mu\text{l}$  of medium containing interleukin-3 or interleukin-3 variant protein once serial dilutions were completed. Control wells contained tissue culture



medium alone (negative control). AML 193 1.3 cell suspensions prepared as above were added to each well by pipetting 50  $\mu$ l ( $2.5 \times 10^4$  cells) into each well. Tissue culture plates were incubated at 37°C with 5% CO<sub>2</sub> in humidified air for 3 days. On day 3, 0.5  $\mu$ Ci <sup>3</sup>H-thymidine (2 Ci/mM, New England Nuclear, Boston, MA) was added in 50  $\mu$ l of tissue culture medium. Cultures were incubated at 37°C with 5% CO<sub>2</sub> in humidified air for 18-24 hours. Cellular DNA was harvested onto glass filter mats (Pharmacia LKB, Gaithersburg, MD) using a TOMTEC cell harvester (TOMTEC, Orange, CT) which utilized a water wash cycle followed by a 70% ethanol wash cycle. Filter mats were allowed to air dry and then placed into sample bags to which scintillation fluid (Scintiverse II, Fisher Scientific, St. Louis, MO or BetaPlate Scintillation Fluid, Pharmacia LKB, Gaithersburg, MD) was added. Beta emissions of samples from individual tissue culture wells were counted in a LKB Betaplate model 1205 scintillation counter (Pharmacia LKB, Gaithersburg, MD) and data was expressed as counts per minute of <sup>3</sup>H-thymidine incorporated into cells from each tissue culture well. Activity of each human interleukin-3 preparation or human interleukin-3 variant preparation was quantitated by measuring cell proliferation (<sup>3</sup>H-thymidine incorporation) induced by graded concentrations of interleukin-3 or interleukin-3 variant. Typically, concentration ranges from 0.05 pM - 10<sup>5</sup> pM are quantitated in these assays. Activity is determined by measuring the dose of interleukin-3 or interleukin-3 variant which provides 50% of maximal proliferation [EC<sub>50</sub> = 0.5 x (maximum average counts per minute of <sup>3</sup>H-thymidine incorporated per well among triplicate cultures of all concentrations of interleukin-3 tested - background proliferation measured by <sup>3</sup>H-thymidine incorporation observed in triplicate cultures lacking interleukin-3)]. This EC<sub>50</sub> value is also equivalent to 1 unit of bioactivity. Every assay was performed with native interleukin-3 as a reference standard so that relative activity levels could be assigned.

Relative biological activities of IL-3 muteins of the present invention are shown in Table 1. The Relative Biological Activity of IL-3 mutants is calculated by dividing the EC<sub>50</sub> of (1-133) hIL-3 by the EC<sub>50</sub> of the mutant. The Relative Biological Activity may be the average of replicate assays.

TABLE 1

10

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

	Plasmid	Polypeptide	Relative*
	Code	Structure	Biological Activity
15	Reference (1-133)hIL-3		1
	pMON13298	SEO ID NO. 82	3
	pMON13299	SEO ID NO. 83	2
	pMON13300	SEO ID NO. 84	3
	pMON13301	SEO ID NO. 85	2
20	pMON13302	SEO ID NO. 86	1.2
	pMON13303	SEO ID NO. 87	0.6
	pMON13287	SEO ID NO. 88	26
	pMON13288	SEO ID NO. 89	24
	pMON13289	SEO ID NO. 90	13
25	pMON13290	SEO ID NO. 91	20
	pMON13292	SEO ID NO. 92	6
	pMON13294	SEO ID NO. 93	3
	pMON13295	SEO ID NO. 94	3
	pMON13312	SEO ID NO. 95	4
30	pMON13313	SEO ID NO. 96	8
	pMON13285	SEO ID NO. 259	32
	pMON13286	SEO ID NO. 260	8
	pMON13325	SEO ID NO. 261	8
	pMON13326	SEO ID NO. 262	25
35	pMON13330	SEO ID NO. 263	19
	pMON13329	SEO ID NO. 406	10
	pMON13364	SEO ID NO. 117	13

TABLE 1 (cont'd)

## BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

5	Plasmid Code	Polypeptide Structure	Relative*
			Biological Activity
	pMON13475	SEO ID NO. 280	7
	pMON13366	SEO ID NO. 281	38
10	pMON13367	SEO ID NO. 282	36
	pMON13368	SEO ID NO. 278	1.6
	pMON13369	SEO ID NO. 283	10
	pMON13370	SEO ID NO. 284	6
	pMON13373	SEO ID NO. 285	12
15	pMON13374	SEO ID NO. 286	6
	pMON13375	SEO ID NO. 287	14
	pMON13376	SEO ID NO. 288	0.4
	pMON13377	SEO ID NO. 289	0.4
	pMON13379	SEO ID NO. 291	0.9
20	pMON13380	SEO ID NO. 279	0.05
	pMON13381	SEO ID NO. 293	10
	pMON13382	SEO ID NO. 313	38
	pMON13383	SEO ID NO. 294	0.5
	pMON13384	SEO ID NO. 295	0.25
25	pMON13385	SEO ID NO. 292	1
	pMON13387	SEO ID NO. 308	32
	pMON13388	SEO ID NO. 296	23
	pMON13389	SEO ID NO. 297	10
	pMON13391	SEO ID NO. 298	30
30	pMON13392	SEO ID NO. 299	17
	pMON13393	SEO ID NO. 300	32
	pMON13394	SEO ID NO. 301	20
	pMON13395	SEO ID NO. 302	11
	pMON13396	SEO ID NO. 303	20
35	pMON13397	SEO ID NO. 304	16
	pMON13398	SEO ID NO. 305	36
	pMON13399	SEO ID NO. 306	18
	pMON13404	SEO ID NO. 307	1.3
	pMON13417	SEO ID NO. 310	24
40	pMON13420	SEO ID NO. 311	19
	pMON13421	SEO ID NO. 312	0.5
	pMON13432	SEO ID NO. 313	10
	pMON13400	SEO ID NO. 317	0.09
	pMON13402	SEO ID NO. 318	20
45	pMON13403	SEO ID NO. 321	0.03
	pMON13405	SEO ID NO. 267	9

TABLE 1 (cont'd)

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

			Relative*
5	Plasmid	Polypeptide	Biological
	Code	Structure	Activity
	pMON13406	SEQ ID NO. 264	5
10	pMON13407	SEQ ID NO. 266	16
	pMON13408	SEQ ID NO. 269	7
	pMON13409	SEQ ID NO. 270	15
	pMON13410	SEQ ID NO. 271	0.4
	pMON13411	SEQ ID NO. 322	1.2
15	pMON13412	SEQ ID NO. 323	0.5
	pMON13413	SEQ ID NO. 324	0.6
	pMON13414	SEQ ID NO. 265	4
	pMON13415	SEQ ID NO. 268	4
	pMON13418	SEQ ID NO. 326	0.5
20	pMON13419	SEQ ID NO. 325	0.015
	pMON13422	SEQ ID NO. 272	0.4
	pMON13423	SEQ ID NO. 273	0.4
	pMON13424	SEQ ID NO. 274	3
	pMON13425	SEQ ID NO. 275	6
25	pMON13426	SEQ ID NO. 276	>0.0003
	pMON13429	SEQ ID NO. 277	>0.0002
	pMON13440	SEQ ID NO. 319	9
	pMON13451	SEQ ID NO. 320	0.1
	pMON13459	SEQ ID NO. 328	0.003
30	pMON13416	SEQ ID NO. 309	19.9
	pMON13428	SEQ ID NO. 327	0.008
	pMON13467	SEQ ID NO. 329	0.16
	pMON13446	SEQ ID NO. 315	21.5
	pMON13390	SEQ ID NO. 316	20

\* The Relative Biological Activity of IL-3 mutants is calculated by dividing the EC50 of (1-133) hIL-3 by the EC50 of the mutant.

The following assay is used to measure IL-3 mediated sulfidoleukotriene release from human mononuclear cells.

IL-3 mediated sulfidoleukotriene release from human mononuclear cells

Heparin-containing human blood was collected and layered onto an equal volume of Ficoll-Paque (Pharmacia #

17-0840-02) ready to use medium (density 1.077 g/ml.). The Ficoll was warmed to room temperature prior to use and clear 50 ml polystyrene tubes were utilized. The Ficoll gradient was spun at 300 x g for 30 minutes at room temperature using a H1000B rotor in a Sorvall RT6000B refrigerated centrifuge. The band containing the mononuclear cells was carefully removed, the volume adjusted to 50 mls with Dulbecco's phosphate-buffered saline (Gibco Laboratories cat. # 310-4040PK), spun at 400 x g for 10 minutes at 4°C and the supernatant was carefully removed. The cell pellet was washed twice with HA Buffer [ 20 mM Hepes (Sigma # H-3375), 125 mM NaCl (Fisher # S271-500), 5 mM KCl (Sigma # P-9541), 0.5 mM glucose (Sigma # G-5000), 0.025% Human Serum Albumin (Calbiochem # 126654) and spun at 300 x g, 10 min., 4°C. The cells were resuspended in HACM Buffer (HA buffer supplemented with 1 mM CaCl<sub>2</sub> (Fisher # C79-500) and 1 mM MgCl<sub>2</sub> (Fisher # M-33) at a concentration of 1 x 10<sup>6</sup> cells/ml and 180 µl were transferred into each well of 96 well tissue culture plates. The cells were allowed to acclimate at 37°C for 15 minutes. The cells were primed by adding 10 µls of a 20 X stock of various concentrations of cytokine to each well (typically 100000, 20000, 4000, 800, 160, 32, 6.4, 1.28, 0 fM IL3). The cells were incubated for 15 minutes at 37°C. Sulfidoleukotriene release was activated by the addition of 10 µls of 20 X (1000 nM) fmet-leu-phe (Calbiochem # 344252) final concentration 50nM FMLP and incubated for 10 minutes at 37°C. The plates were spun at 350 x g at 4°C for 20 minutes. The supernatants were removed and assayed for sulfidoleukotrienes using Cayman's Leukotriene C4 EIA kit (Cat. #420211) according to manufacturers' directions. Native (15-125)hIL-3 was run as a standard control in each assay.

Native hIL-3 possesses considerable inflammatory activity and has been shown to stimulate synthesis of the arachidonic acid metabolites LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>; histamine synthesis and histamine release. Human clinical

trials with native hIL-3 have documented inflammatory responses (Biesma, et al., BLOOD, 80:1141-1148 (1992) and Postmus, et al., J. CLIN. ONCOL., 10:1131-1140 (1992)). A recent study indicates that leukotrienes are involved in IL-3 actions in vivo and may contribute significantly to the biological effects of IL-3 treatment (Denzlinger, C., et al., BLOOD, 81:2466-2470 (1993))

Some muteins of the present invention may have an improved therapeutic profile as compared to native hIL-3 or (15-125)hIL-3. For example, some muteins of the present invention may have a similar or more potent growth factor activity relative to native hIL-3 or (15-125)hIL-3 without having a similar or corresponding increase in the stimulation of leukotriene or histamine. These muteins would be expected to have a more favorable therapeutic profile since the amount of polypeptide which needs to be given to achieve the desired growth factor activity (e. g. cell proliferation) would have a lesser leukotriene or histamine stimulating effect. In studies with native hIL-3, the stimulation of inflammatory factors has been an undesirable side effect of the treatment. Reduction or elimination of the stimulation of mediators of inflammation would provide an advantage over the use of native hIL-3.

The pMON13288 polypeptide has demonstrated a more potent growth factor activity relative to native hIL-3 in the AML 193 cell proliferation assay ( $EC_{50} = 0.8 - 3.8$  pM for pMON13288 and  $EC_{50} = 30.2$  pM for native hIL-3) without demonstrating a corresponding increase in the stimulation of leukotriene C<sub>4</sub> (LTC<sub>4</sub>) production and histamine release, i. e., it stimulated LTC<sub>4</sub> production and histamine release with a potency similar to that of native hIL-3 while having an improved ability to stimulate cell proliferation compared to native hIL-3. Thus with the pMON13288 polypeptide it would be expected that one would be able to produce a desired therapeutic response, e. g., cell proliferation, with less stimulation of the undesirable inflammatory mediators.

Some muteins of the present invention have antigenic

profiles which differ from that of native hIL-3. For example, in a competition ELISA with an affinity purified polyclonal goat anti-hIL-3 antibody, native hIL-3 significantly blocked the binding of labeled hIL-3 to polyclonal anti-hIL-3 antibody whereas the pMON13288 polypeptide failed to block the binding of hIL-3 to anti-hIL-3 antibody.

Table 2 lists the sequences of some oligonucleotides used in making the muteins of the present invention.

Table 3 lists the amino acid sequence of native (15-125)hIL-3 (Peptide #1) and the amino acid sequences of some mutant polypeptides of the present invention. The sequences are shown with the amino acid numbering corresponding to that of native hIL-3 [FIG. 1].

Table 4 lists the nucleotide sequences of some DNA sequences which encode mutant polypeptides of the present invention.

#### TABLE 2

#### OLIGONUCLEOTIDES

Oligo #1 Length: 000040

CATGGCTAAC TGCTCTATAA TGATCGATGA AATTATACAT [SEQ ID NO:15]

Oligo #2 Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATCAT TATAGAGCAG TTAGC

[SEQ ID NO:16]

Oligo #3 Length: 000036

CACTTAAAGA GACCACCTGC ACCTTTGCTG GACCCG [SEQ ID NO:17]

Oligo #4 Length: 000036

GAGGTTGTTC GGGTCCAGCA AAGGTGCAGG TGGTCT [SEQ ID NO:18]

Oligo #5 Length: 000036

CACTTAAAGA GACCACCTAA CCCTTTGCTG GACCCG [SEQ ID NO:19]

Oligo #6 Length: 000036  
GAGGTTGTTC GGGTCCAGCA AAGGGTTAGG TGGTCT [SEQ ID NO:20]

5 Oligo #7 Length: 000036  
CACTTAAAGG TTCCACCTGC ACCTTTGCTG GACAGT [SEQ ID NO:21]

Oligo #8 Length: 000036  
GAGGTTGTTA CTGTCCAGCA AAGGTGCAGG TGGAAC [SEQ ID NO:22]

10 Oligo #9 Length: 000027  
AACCAACCTCA ATGCTGAAGA CGTTGAT [SEQ ID NO:23]

Oligo #10 Length: 000018  
ATCAACGTCT TCAGCATT [SEQ ID NO:24]

15 Oligo #11 Length: 000027  
AACCAACCTCA ATTCTGAAGA CATGGAT [SEQ ID NO:25]

Oligo #12 Length: 000018  
20 ATCCATGTCT TCAGAATT [SEQ ID NO:26]

Oligo #13 Length: 000022  
CATGGGAACC ATATGTCAGG AT [SEQ ID NO:27]

25 Oligo #14 Length: 000018  
ATCCTGACAT ATGGTTCC [SEQ ID NO:28]

Oligo #15 Length: 000016  
TGAACCATAT GTCAGG [SEQ ID NO:29]

30 Oligo #16 Length: 000024  
AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:30]

Oligo #17 Length: 000020  
35 AATTCGAACC ATATGTCAGA [SEQ ID NO:31]

Oligo #18 Length: 000020  
AGCTTCTGAC ATATGGTTCG [SEQ ID NO:32]



Oligo #19 Length: 000022  
ATCGAACCAT ATGTCAGATG CA [SEQ ID NO:33]

5 Oligo #20 Length: 000018  
TCTGACATAT GGTTCGAT [SEQ ID NO:34]

Oligo #21 Length: 000036  
ATCCTGATGG AACGAAACCT TCGACTTCCA AACCTG [SEQ ID NO:35]

10 Oligo #22 Length: 000027  
AAGTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:36]

Oligo #23 Length: 000036  
15 ATCCTGATGG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:37]

Oligo #24 Length: 000027  
AGTTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:38]

20 Oligo #25 Length: 000024  
CTCGCATTCG TAAGGGCTGT CAAG [SEQ ID NO:39]

Oligo #26 Length: 000024  
CCTTACGAAT GCGAGCAGGT TTGG [SEQ ID NO:40]

25 Oligo #27 Length: 000024  
GAGAGCTTCG TAAGGGCTGT CAAG [SEQ ID NO:41]

30 Oligo #28 Length: 000024  
CCTTACGAAG CTCTCCAGGT TTGG [SEQ ID NO:42]

Oligo #29 Length: 000015  
CACTTAGAAA ATGCA [SEQ ID NO:43]

35 Oligo #30 Length: 000020  
TTTTCTAAGT GCTTGACAGC [SEQ ID NO:44]

Oligo #31 Length: 000015  
AACTTAGAAA ATGCA [SEQ ID NO:45]

5 Oligo #32 Length: 000020  
TTTTCTAAGT TCTTGACAGC [SEQ ID NO:46]

10 Oligo #33 Length: 000048  
GGTGATTGGA TGTCGAGAGG GTGCGGCCGT GGCAGAGGGC AGACATGG  
[SEQ ID NO:47]

Oligo #34 Length: 000048  
CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA CCATCAAG  
[SEQ ID NO:48]

15 Oligo #35 Length: 000048  
GATGATTGGA TGTCGAGAGG GTGCGGCCGT GGCAGAGGGC AGACATGG  
[SEQ ID NO:49]

20 Oligo #36 Length: 000048  
CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA TCATCAAG  
[SEQ ID NO:50]

Oligo #37 Length: 000018  
TACGAGATTA CGAAGAAT [SEQ ID NO:51]

25 Oligo #38 Length: 000018  
CGTAATCTCG TACCATGT [SEQ ID NO:52]

Oligo #39 Length: 000018  
30 TTGGAGATTA CGAAGAAT [SEQ ID NO:53]

Oligo #40 Length: 000018  
CGTAATCTCC AACCATGT [SEQ ID NO:54]

35 Oligo #41 Length: 000019  
TGCCTCAATA CCTGATGCA [SEQ ID NO:55]

- Oligo #42 Length: 000021  
TCAGGTATTG AGGCAATTCT T [SEQ ID NO:56]
- 5 Oligo #43 Length: 000026  
AATTCTTGCC AGTCACCTGC CTTGAT [SEQ ID NO:57]
- Oligo #44 Length: 000016  
GCAGGTGACT GGCAAG [SEQ ID NO:58]
- 10 Oligo #45 Length: 000032  
AATTCCGGGA AAAACTGACG TTCTATCTGG TT [SEQ ID NO:59]
- Oligo #46 Length: 000037  
CTCAAGGGAA ACCAGATAGA ACGTCAGTTT TTCCCGG [SEQ ID NO:60]
- 15 Oligo #47 Length: 000032  
ACCCCTTGAGC ACGCGCAGGA ACAACAGTAA TA [SEQ ID NO:61]
- Oligo #48 Length: 000027  
20 AGCTTATTAC TGTGTTCCT GCGCGTG [SEQ ID NO:62]
- Oligo #49 Length: 000032  
ACCCCTTGAGC AAGCGCAGGA ACAACAGTAA TA [SEQ ID NO:63]
- 25 Oligo #50 Length: 000027  
AGCTTATTAC TGTGTTCCT GCGCTTG [SEQ ID NO:64]
- Oligo #51 Length: 000034  
GCCGATACCGCGGCATACTCCACCATTCAGAGA [SEQ ID NO:155]
- 30 Oligo #52 Length: 000033  
GCCGATAAGATCTAAAACGGGTATGGAGAAACA [SEQ ID NO:156]
- Oligo #53  
35 ATAGTCTTCCCCAGATATCTAACGCTTGAG [SEQ ID NO:157]
- Oligo #54 Length: 24  
CAATACCTGATGCGTTTTCTAAGT [SEQ ID NO:158]
- 40 Oligo #55 Length: 33  
GGTTTCGTTCCATCAGAATGTCCATGTCTTCAG [SEQ ID NO:159]

Oligo #165 NCOECRV1.REQ Length: 000040

CATGGCTAAC TGCTCTAACA TGATCGATGA AATTATAACA [SEQ ID NO:162]

5 Oligo #166 NCOECRV4.REQ Length: 000045

CTTTAAGTGT GTTATAATTT CATCGATCAT GTTAGAGCAG TTAGC [SEQ ID NO:163]

10 Oligo #167 NCOECRV2.REQ Length: 000036

CACTTAAAGC AGCCACCTTT GCCTTTGCTG GACTTC [SEQ ID NO:164]

15 Oligo #168 NCOECRV5.REQ Length: 000036

GAGGTTGTTG AAGTCCAGCA AAGGCAAAGG TGGCTG [SEQ ID NO:165]

Oligo #169 2D5M6SUP.REQ Length: 000027

20 AACAACTCA ATGACGAAGA CATGTCT [SEQ ID NO:166]

Oligo #170 2D5M6SLO.REQ Length: 000018

25 AGACATGTCT TCGTCATT [SEQ ID NO:167]

Oligo #15(A) Length: 000016

TGAACCATAT GTCAGG [SEQ ID NO:168]

30 Oligo #16(A) Length: 000024

AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:169]

Oligo #B1 19ALA1.REQ Length: 000040  
CATGGCAAAC TGCTCTATAG CTATCGATGA AATTATACAT [SEQ ID NO:170]

5 Oligo #B2 19ALA4.REQ Length: 000045  
CTTTAAGTGA TGTATAATTT CATCGATAGC TATAGAGCAG TTTGC [SEQ ID  
NO:171]

10 Oligo #B3 19ILE1.REQ Length: 000040  
CATGGCAAAC TGCTCTATAA TCATCGATGA AATTATACAT [SEQ ID NO:172]

Oligo #B4 19ILE4.REQ Length: 000045  
15 CTTTAAGTGA TGTATAATTT CATCGATGAT TATAGAGCAG TTTGC [SEQ ID  
NO:173]

Oligo #B5 49ASP1.REQ Length: 000036  
20 ATCCTGGACG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:174]

Oligo #B6 49ASP4.REQ Length: 000027  
25 AGTTCGAAGG TTTCGTTTCGT CCAGGAT [SEQ ID NO:175]

Oligo #B7 49ILE1.REQ Length: 000036  
ATCCTGATCG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:176]

30 Oligo #B8 49ILE4.REQ Length: 000027  
AGTTCGAAGG TTTCGTTTCGA TCAGGAT [SEQ ID NO:177]

35 Oligo #B9 49LEU1.REQ Length: 000036  
ATCCTGCTGG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:178]

Oligo #B10 49LEU4.REQ Length: 000027  
40 AGTTCGAAGG TTTCGTTCCA GCAGGAT [SEQ ID NO:179]

Oligo #B11 42S45V3.REQ Length: 000027  
45 AACAACTCA ATTCTGAAGA CGTTGAT [SEQ ID NO:180]

Oligo #B12 42S45V6.REQ Length: 000018  
ATCAACGTCT TCAGAATT [SEQ ID NO:181]

50 Oligo #B13 18I23A5H.REQ Length: 000051  
CGCGCCATGG CTAACGCTC TATAATGATC GATGAAGCAA TACATCACTTA  
[SEQ ID NO:182]

55 Oligo #B14 2341HIN3.REQ Length: 000018  
CGCGTCGATA AGCTTATT [SEQ ID NO:183]

60 Oligo #B15 2341NCO.REQ Length: 000018  
GGAGATATAT CCATGGCT [SEQ ID NO:184]

Oligo #B16            2A5M6S0D.REQ   Length: 000042  
TCGGTCCATC AGAATAGACA TGTCTTCAGC ATTGAGGTTG TT [SEQ ID NO:185]  
5 Oligo #B17            2A5V6S0D.REQ   Length: 000042  
TCGGTCCATC AGAATAGAAA CGTCTTCAGC ATTGAGGTTG TT [SEQ ID NO:186]  
10 Oligo #B18            2D5M6S0D.REQ   Length: 000042  
TCGGTCCATC AGAATAGACA TGTCTTCGTC ATTGAGGTTG TT [SEQ ID NO:187]  
Oligo #B19            2D5V6S0D.REQ   Length: 000042  
15 TCGGTCCATC AGAATAGAAA CGTCTTCGTC ATTGAGGTTG TT [SEQ ID NO:188]  
Oligo #B20            2S5M6S0D.REQ   Length: 000042  
20 TCGGTCCATC AGAATAGACA TGTCTTCAGA ATTGAGGTTG TT [SEQ ID NO:189]  
Oligo #B21            2S5V6S0D.REQ   Length: 000042  
TCGGTCCATC AGAATAGAAA CGTCTTCAGA ATTGAGGTTG TT [SEQ ID NO:190]  
25 Oligo #B22            100ARG3.REQ   Length: 000048  
CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA TCATCCGT [SEQ ID  
NO:191]  
30 Oligo #B23            100ARG8.REQ   Length: 000026  
AATTCTTGCC AGTCACCTGC ACGGAT [SEQ ID NO:192]  
35 Oligo #B24            101MET4.REQ   Length: 000016  
ATGGGTGACT GGCAAG [SEQ ID NO:193]  
Oligo #B25            10R01M8.REQ   Length: 000026  
40 AATTCTTGCC AGTCACCCAT ACGGAT [SEQ ID NO:194]  
Oligo #B26            23ALA1.REQ   Length: 000040  
45 CATGGCTAAC TGCTCTATTA TGATCGATGA AGCAATACAT [SEQ ID NO:195]  
Oligo #B27            23ALA4.REQ   Length: 000045  
CTTTAAGTGA TGTATTGCTT CATCGATCAT AATAGAGCAG TTAGC [SEQ ID  
50 NO:196]  
Oligo #B28            29V2R4S2.REQ   Length: 000036  
CACTTAAAGG TACCACCTCG CCCTTCCCTG GACCCG [SEQ ID NO:197]  
55 Oligo #B29            29V2R4S5.REQ   Length: 000036  
GAGGTTGTTC GGGTCCAGGG AAGGGCGAGG TGGTAC [SEQ ID NO:198]  
60 Oligo #B30            34SER2.REQ   Length: 000036  
CACTTAAAGA GACCACCTGC ACCTTCCCTG GACCCG [SEQ ID NO:199]

Oligo #B31 34SER5.REQ Length: 000036  
GAGGTTGTTC GGGTCCAGGG AAGGTGCAGG TGGTCT [SEQ ID NO:200]  
5  
Oligo #B32 42D45M3.REQ Length: 000027  
AACCAACCTCA ATGACGAAGA CATGGAT [SEQ ID NO:201]  
10  
Oligo #B33 42D45M6.REQ Length: 000018  
ATCCATGTCT TCGTCATT [SEQ ID NO:202]  
Oligo #B34 42D45V3.REQ Length: 000027  
15  
AACCAACCTCA ATGACGAAGA CGTCGAT [SEQ ID NO:203]  
Oligo #B35 42D45V6.REQ Length: 000018  
20  
ATCGACGTCT TCGTCATT [SEQ ID NO:204]  
Oligo #B36 42D5M6S3.REQ Length: 000027  
AACCAACCTCA ATGACGAAGA CATGTCT [SEQ ID NO:205]  
25  
Oligo #B37 42D5M6S6.REQ Length: 000018  
AGACATGTCT TCGTCATT [SEQ ID NO:206]  
30  
Oligo #B38 42D5V6S3.REQ Length: 000027  
AACCAACCTCA ATGACGAAGA CGTCTCT [SEQ ID NO:207]  
Oligo #B39 42D5V6S6.REQ Length: 000018  
35  
AGAGACGTCT TCGTCATT [SEQ ID NO:208]  
Oligo #B40 50ASP1.REQ Length: 000036  
40  
ATCCTGATGG ACCGAAACCT TCGACTTCCA AACCTG [SEQ ID NO:209]  
Oligo #B41 50ASP4.REQ Length: 000027  
AAGTCGAAGG TTTCGGTCCA TCAGGAT [SEQ ID NO:210]  
45  
Oligo #B42 50D56S1.REQ Length: 000036  
ATCCTGATGG ACCGAAACCT TCGACTTAGC AACCTG [SEQ ID NO:211]  
50  
Oligo #B43 56SER5.REQ Length: 000024  
CCTTACGAAG CTCTCCAGGT TGCT [SEQ ID NO:212]  
Oligo #B44 82TRP2.REQ Length: 000018  
55  
CGTAATCTCT GGCCATGT [SEQ ID NO:213]  
Oligo #B45 82TRP6.REQ Length: 000018  
60  
CCAGAGATTA CGAAGAAT [SEQ ID NO:214]

Oligo #B46 9E12Q6W1.REQ Length: 000032  
AATTCCGGGA AAAACTGCAA TTCTATCTGT GG [SEQ ID NO:215]

5 Oligo #B47 9E12Q6W3.REQ Length: 000037  
CTCAAGGGTC CACAGATAGA ATTGCAGTTT TTCCCGG [SEQ ID NO:216]

10 Oligo #B48 9E12Q6V1.REQ Length: 000032  
AATTCCGGGA AAAACTGCAA TTCTATCTGG TT [SEQ ID NO:217]

Oligo #B49 9E12Q6V3.REQ Length: 000037  
15 CTCAAGGGTA ACCAGATAGA ATTGCAGTTT TTCCCGG [SEQ ID NO:218]

Oligo #B50 S09E16V1.REQ Length: 000023  
AATTCCGGGA AAAACTGACG TTC [SEQ ID NO:219]

20 Oligo #B51 S09E16V3.REQ Length: 000028  
AACCAGATAG AACGTCAGTT TTTCCCGG [SEQ ID NO:220]

25 Oligo #B52 S116VD31.REQ Length: 000023  
TATCTGGTTA CCCTTGAGTA ATA [SEQ ID NO:221]

Oligo #B53 SECR1D33.REQ Length: 000018  
30 AGCTTATTAC TTCAAGGGT [SEQ ID NO:222]

Oligo #B54 S9E2Q6V1.REQ Length: 000023  
35 AATTCCGGGA AAAACTGCAA TTC [SEQ ID NO:223]

Oligo #B55 S9E2Q6V3.REQ Length: 000028  
AACCAGATAG AATTGCAGTT TTTCCCGG [SEQ ID NO:224]

40 Oligo #B56 Ent338.Lo Length: 61  
CGATCATTAT AGAGCAGTTA GCCTTGTCAT CGTCGTCCTT GTAATCAGTT  
TCTGGATATG C [SEQ ID NO:225]

45 Oligo #B57 Ent338.UP Length: 63  
CATGGCATAT CCAGAACTG ATTACAAGGA CGACGATGAC AAGGCTAACT  
GCTCTATAAT GAT [SEQ ID NO:226]

50 09L2Q6S1.REQ Length: 000032  
AATTCCGGCT TAAACTGCAA TTCTATCTGT CT [SEQ ID NO:227]

55 09L2Q6S3.REQ Length: 000037  
CTCAAGGGTA GACAGATAGA ATTGCAGTTT AAGCCGG [SEQ ID NO:228]



117S2.REQ Length: 000032  
TCTCTTGAGC AAGCGCAGGA ACAACAGTAA TA [SEQ ID NO:229]

5 19I0L3A1.REQ Length: 000040  
CATGGCAAAC TGCTCTATAA TACTCGATGA AGCAATACAT [SEQ ID NO:230]

10 19I0L3A4.REQ Length: 000045  
CTTTAAGTGA TGTATTGCTT CATCGAGTAT TATAGAGCAG TTTGC [SEQ. ID NO.:231]

15 20P23A1.REQ Length: 000040  
CATGGCAAAC TGCTCTATAA TGCCAGATGA AGCAATACAT [SEQ. ID NO.:232]

20 20P23A4.REQ Length: 000045  
CTTTAAGTGA TGTATTGCTT CATCTGGCAT TATAGAGCAG TTTGC [SEQ. ID NO.:233]

25 23L1.REQ Length: 000040  
CATGGCaAAC TGCTCTATAA TGATCGATGA AactgATACAT [SEQ. ID NO.:234]

30 23L4.REQ Length: 000045  
CTTTAAGTGA TGTATcagTT CATCGATCAT TATAGAGCAG TTtGC [SEQ. ID NO.:235]

35 29I4S7S2.REQ Length: 000036  
CACTTAAAGA TACCACCTAA CCCTAGCCTG GACAGT [SEQ. ID NO.:236]

40 29I4S7S5.REQ Length: 000036  
GAGGTTAGCA CTGTCCAGGC TAGGGTTAGG TGGTAT [SEQ. ID NO.:237]

45 38A5V6S3.REQ Length: 000027  
GCTAACCTCA ATTCCGAAGA CGTCTCT [SEQ. ID NO.:238]

50 38A5V6S6.REQ Length: 000018  
AGAGACGTCT TCGGAATT [SEQ. ID NO.:239]

55 50D51S1.REQ Length: 000036  
ATCCTGATGG ACTCCAACCT TCGAACTCCA AACCTG [SEQ. ID NO.:240]

60 50D51S4.REQ Length: 000027  
AGTTCGAAGG TTGGAGTCCA TCAGGAT [SEQ. ID NO.:241]

SVYWPTT3.REQ Length: 000048  
GTTCCCTATT GGACGGCCCC TCCCTCTCGA ACACCAATCA CGATCAAG [SEQ. ID NO.:242]

5VYWPTT7.REQ Length: 000048  
CGTGATTGGT GTTCGAGAGG GAGGGGCCGT CCAATAGGGA ACACATGG [SEQ. ID  
5 NO.:243]  
62P3H5S2.REQ Length: 000024  
CTCGCATTCC CACATGCTTC TAAG [SEQ. ID NO.:244]  
10 62P63H2.REQ Length: 000024  
CTCGCATTCC CACATGCTGT CAAG [SEQ. ID NO.:245]  
15 62P63H5.REQ Length: 000024  
ATGTGGGAAT GCGAGCAGGT TTGG [SEQ. ID NO.:246]  
65S67Q6.REQ Length: 000020  
20 TTTTCTAATT GCTTAGAAGC [SEQ. ID NO.:247]  
67Q3.REQ Length: 000015  
25 CAATTAGAAA ATGCA [SEQ. ID NO.:248]  
67Q6.REQ Length: 000021  
TTTTCTAATT GCTTGACAGC [SEQ. ID NO.:249]  
30 76P1.REQ Length: 000021  
TCAGGTATTG AGCCAATTCT T [SEQ. ID NO.:250]  
35 76P5.REQ Length: 000019  
TGGCTCAATA CCTGATGCA [SEQ. ID NO.:251]  
79S2.REQ Length: 000018  
40 TCTAATCTCC AACCATGT [SEQ. ID NO.:252]  
79S6.REQ Length: 000018  
45 TTGGAGATTA GAAAGAAT [SEQ. ID NO.:253]  
9L2Q67S3.REQ Length: 000037  
CTCAAGAGAA GACAGATAGA ATTGCAGTTT AAGCCGG [SEQ. ID NO.:254]  
50 9LQS1181.REQ Length: 000043  
AATTCCGGCT TAAACTGCAA TTCTATCTGT CTACCCTTTA ATA [SEQ. ID  
55 NO.:256]  
9LQS1183.REQ Length: 000043  
AGCTTATTAA AGGGTAGACA GATAGAATTG CAGTTTAAGC CGG [SEQ. ID  
60 NO.:257]

S9L2Q6S1.REQ

Length: 000043

AATTCCGGCT TAAACTGCAA TTCTATCTGT CTACCCTTTA ATA [SEQ. ID  
NO.:258]

5

TABLE 3

10

POLYPEPTIDES

PEPTIDE #1; pMON5988 (Example 43); (15-125)hIL-3

15	Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu	15	20	25
	Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly	30	35	40
20	Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn	45	50	55
	Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser	60	65	70
25	Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu	75	80	85
	Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly	90	95	100
30	Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr	105	110	115
35	Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:65]	120	125	
	Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu			
40	Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly			
	Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn			
45	Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser			
	Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu			
50	Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly			
55	Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr			
	Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:65]			
60				

**PEPTIDE #2;** pMON13344 (Example 8); (15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A and 45V);

5 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
30 35 40

10 Glu Asp Val Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
45 50 55

15 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 80 85

20 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
105 110 115

25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:66]  
120 125

30 **PEPTIDE #3;** pMON13345 (Example 9); (15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S and 45M);

35 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

40 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
60 65 70

45 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
90 95 100

50 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
105 110 115

55 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:67]  
120 125

60 **PEPTIDE #4;** pMON13346 (Example 10); (15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S and 45M);

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

5 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
 30 35 40

10 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70

15 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100

20 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115

25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:68]  
 120 125

**PEPTIDE #5; pMON13347 (Example 12); (15-125)hIL-3 (51R, 55L, 59L, 62V, 67N and 69E);**

30

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25

35 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 30 35 40

Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

40 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85

45 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100

50 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115

55 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]  
 120 125

**PEPTIDE #6; pMON13348 (Example 13); (15-125)hIL-3 (51R, 55L, 60S, 62V, 67N and 69E);**

60

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

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Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 5 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 10  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 15 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:74]  
 120 125  
 20  
 PEPTIDE #11; pMON13354 (Example 20); (15-125)hIL-3 (109E, 116V,  
 117S, 120H  
 and 123E);  
 25  
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 30 30 35 40  
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 35 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 40 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 105 110 115  
 45 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:75]  
 120 125  
 50 PEPTIDE #12; pMON13360 (Example 21); (15-125)hIL-3 (73G, 76A, 79R,  
 82Q,  
 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);  
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 55 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 30 35 40  
 60 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn



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5 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
60 65 70

10 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ. NO:76]  
120 125

30 **PEPTIDE #13:** pMON13361 (Example 22); (15-125)hIL-3 (73G, 76A, 79R,  
82V,  
87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);

35 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
15 20 25

40 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
30 35 40

45 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
45 50 55

50 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
75 80 85

60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
90 95 100

65 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

70 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:77]  
120 125

75 **PEPTIDE #14:** pMON13362 (Example 23); (15-125)hIL-3 (73G, 76A, 79R,  
82V,  
87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and  
123E);

80 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
15 20 25

85 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
30 35 40

90 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
45 50 55

5 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 75 80 85  
 10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 105 110 115  
 15 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:78]  
 120 125  
 PEPTIDE #15; pMON13363 (Example 24); (15-125)hIL-3 (18I, 25H, 29R,  
 32A,  
 20 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E);  
 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 25 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 30 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 35 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 40 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115  
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:79]  
 120 125  
 45  
 PEPTIDE #16; pMON13364 (Example 25); (15-125)hIL-3 (18I, 25H, 29R,  
 32N,  
 50 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E);  
 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 55 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 60 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 85

5 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
105 110 115

10 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:80]  
120 125

15 **PEPTIDE #17; pMON13365 (Example 26); (15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E);**

20 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
30 35 40

25 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

30 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 80 85

35 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
105 110 115

40 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:81]  
120 125

45 **PEPTIDE #18; pMON13298 (Example 27); Met-Ala-(15-125)hIL-3 (73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);**

50 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
30 35 40

55 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
60 65 70

60 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

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5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:82]  
 120 125  
 PEPTIDE #19; pMON13299 (Example 28); Met-Ala-(15-125)hIL-3 (73G,  
 15 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and  
 123E);  
 20 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 30 35 40  
 25 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 75 80 85  
 35 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 40 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:83]  
 120 125  
 PEPTIDE #20; pMON13300 (Example 29); Met-Ala-(15-125)hIL-3 (73G,  
 45 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H  
 and 123E);  
 50 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 30 35 40  
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 55 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 75 80 85  
 60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

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Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 105 110 115  
 5 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:84]  
 120 125

10 PEPTIDE #21; pMON13301 (Example 30); Met-Ala-(15-125)hIL-3 (18I,  
 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E);  
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 15 20 25  
 15 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 20 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 25 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 30 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115  
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:85]  
 120 125  
 35

PEPTIDE #22; pMON13302 (Example 31); Met-Ala-(15-125)hIL-3 (18I,  
 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E);  
 40 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 45 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 50 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 55 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 60 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:86]  
120 125

5 **PEPTIDE #23**; pMON13303 (Example 32); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E);

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

10 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
30 35 40

15 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

20 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
90 95 100

25 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
105 110 115

30 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:87]  
120 125

35 **PEPTIDE #24**; pMON13287 (Example 33); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

40 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

45 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:88]  
120 125

60

**PEPTIDE #25;** pMON13288 (Example 34); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

10 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:89]  
120 125

30 **PEPTIDE #26;** pMON13289 (Example 35); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

40 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

45 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:90]  
120 125

60 **PEPTIDE #27;** pMON13290 (Example 36); Met-Ala-(15-125)hIL-3 (18I,

25H, 29R, 37P, 42A, 45V, 51R, 55L, 60S, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

10 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

15 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
90 95 100

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:91]  
120 125

30 **PEPTIDE #28; pMON13292 (Example 37); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);**

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

40 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

45 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:92]  
120 125

60 **PEPTIDE #29; pMON13294 (Example 38); Met-Ala-(15-125)hIL-3 (18I,**



25H, 29R, 37P, 42S, 45M, 51R, 55T, 59L, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E);

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
75 80 85

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
105 110 115

25 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:93]  
120 125

30 **PEPTIDE #30; pMON13295 (Example 39); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E);**

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
30 35 40

40 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
105 110 115

55 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:94]  
120 125

60 **PEPTIDE #31; pMON13312 (Example 40); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and**

123E);

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 25  
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 40  
 10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 55  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 70  
 15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 75 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 90 100  
 20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 115  
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:95]  
 120 125

30 **PEPTIDE #32;** pMON13313 (Example 41); Met-Ala-(15-125)hIL-3 (18I,  
 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G,  
 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H  
 and 123E);

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 25  
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 40  
 40 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 70  
 45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 75 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 90 100  
 50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 105 115  
 55 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:96]  
 120 125

60 **PEPTIDE #A3;** pMON13285 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 50D);

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

15

20

25

5 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Met Ser Ile Leu Met Asp Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 10 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 15 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115  
 20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:259]  
 120 125  
 25 **PEPTIDE #A4;** pMON13286 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S);  
 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 30 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Met Ser Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 35 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 40 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 45 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 105 110 115  
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:260]  
 120 125  
 50 **PEPTIDE #A5;** pMON13325 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S,  
 116W);  
 55 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp  
 30 35 40  
 60 Glu Asp Met Ser Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

45 50 55  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 5 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Trp Thr  
 105 110 115  
 15 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:261]  
 120 125  
 20 **PEPTIDE #A6;** pMON13326 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 50D,  
 116W);  
 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 25 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Met Ser Ile Leu Met Asp Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 30 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70  
 35 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 75 80 85  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 90 95 100  
 40 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Trp Thr  
 105 110 115  
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:262]  
 120 125  
 45  
 50 **PEPTIDE #A7;** pMON13330 Met-Ala-IL-3; (42D, 45M, 46S, 50D, 95R, 98I,  
 100R, 116W);  
 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 15 20 25  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp  
 30 35 40  
 55 Glu Asp Met Ser Ile Leu Met Asp Asn Asn Leu Arg Arg Pro Asn  
 45 50 55  
 60 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 85

5 Ala Thr Ala Ala Pro Thr Arg Arg Pro Ile Ile Ile Arg Asp Gly  
90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Trp Thr  
105 110 115

10 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:263]  
120 125

15 **PEPTIDE #A8; pMON13329 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 98I, 100R, 116W);**

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
15 20 25

20 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Asp  
30 35 40

25 Glu Asp Met Ser Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
45 50 55

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
60 65 70

30 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
75 80 85

Ala Thr Ala Ala Pro Thr Arg His Pro Ile Ile Ile Arg Asp Gly  
90 95 100

35 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Trp Thr  
105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:406]  
120 125

40

**PEPTIDE #B1 Met-Ala-(15-125)hIL-3 pMON13406**

45 Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

50 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

55 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 264]  
 120 125

PEPTIDE # B2 Met-Ala-(15-125)hIL-3 pMON13414

10 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu  
 15 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 15 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 20 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 25 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 265]  
 120 125

35 PEPTIDE #B3 Met-Ala-(15-125)hIL-3 pMON13407

40 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 40 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 45 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 50 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 55 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 60 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 266]  
 120 125

## PEPTIDE #B4 Met-Ala-(15-125)hIL-3 pMON13405

5 Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

10 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 267]  
120 125

## PEPTIDE #B5 Met-Ala-(15-125)hIL-3 pMON13415

30 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
35 30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

40 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 268]  
120 125

## PEPTIDE #B6 Met-Ala-(15-125)hIL-3 pMON13408

55 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

60 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

20

## 25

50

## 55

50	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20					25		
55	Lys	Arg	Pro	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser
			30					35					40		
	Glu	Asp	Met	Asp	Ile	Leu	Asp	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
60	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		



Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 271]  
 120 125

15 PEPTIDE #B9 Met-Ala-(15-125)hIL-3 pMON13422

Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu  
 15 20 25

20 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40

Glu Asp Val Asp Ile Leu Ile Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55

25 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 272]  
 120 125

40

PEPTIDE #B10 Met-Ala-(15-125)hIL-3 pMON13423

Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu  
 15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40

50 Glu Asp Val Asp Ile Leu Ile Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

60

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Leu Val Thr  
 105 110 115

5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 273]  
 120 125

10 PEPTIDE #B11 Met-Ala-(15-125)hIL-3 pMON13424

Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu  
 15 20 25

15 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40

Glu Asp Val Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55

20 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 274]  
 120 125

35 PEPTIDE #B12 Met-Ala-(15-125)hIL-3 pMON13425

Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu  
 15 20 25

40 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40

45 Glu Asp Val Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70

50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

60 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 275]  
 120 125

## PEPTIDE #B13 Met-Ala-(15-125)hIL-3 pMON13426

5 Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

10 Glu Asp Val Asp Ile Leu Asp Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 276]  
120 125

30

## PEPTIDE #B14 Met-Ala-(15-125)hIL-3 pMON13429

35 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

40 Glu Asp Val Asp Ile Leu Asp Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

55 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 277]  
120 125

## PEPTIDE #B15 Met-Ala-(15-125)hIL-3 pMON13368

60 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
15 20 25

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
 30 35 40  
 5 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 10 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 15 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 20 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO: 278]  
 120 125  
 PEPTIDE #B16 Met-Ala-(15-125)hIL-3 pMON13380  
 25 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
 30 30 35 40  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 35 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 40 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
 45 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO. 279]  
 120 125  
 50 PEPTIDE #B17 Met-Ala-(15-125)hIL-3 pMON13475  
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 55 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 60 45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Asn Ala Ser  
 60 65 70  
 5 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 280]  
 120 125

15

PEPTIDE #B18 Met-Ala-(15-125)hIL-3 pMON13366

20 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asn  
 30 35 40  
 25 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 40 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 281]  
 120 125

45 PEPTIDE #B19 Met-Ala-(15-125)hIL-3 pMON13367  
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

50 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 55 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

90

95

100

5 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 282]  
 120 125

## 10 PEPTIDE #B20 Met-Ala-(15-125)hIL-3 pMON13369

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 15 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 20 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 283]  
 120 125

35

## PEPTIDE #B21 Met-Ala-(15-125)hIL-3 pMON13370

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 40 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 45 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 60 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 284]  
 120 125

## PEPTIDE #B22 Met-Ala-(15-125)hIL-3 pMON13378

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
30 35 40

10 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
45 50 55

15 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 285]  
120 125

## 30 PEPTIDE #B23 Met-Ala-(15-125)hIL-3 pMON13374

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

35 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
45 50 55

40 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 286]  
120 125

55

## PEPTIDE #B24 Met-Ala-(15-119)hIL-3 pMON13375

60 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Leu Asn Ala  
 30 35 40  
 5 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 10 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 20 Leu Glu [SEQ ID NO.: 287]  
 119

## PEPTIDE #B25 Met-Asp-(15-119)hIL-3 pMON13376

25 Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 30 35 40  
 30 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 35 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 40 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
 105 110 115  
 45 Leu Glu [SEQ ID NO.: 288]  
 119

## 50 PEPTIDE #B26 Met-Ala-(15-125)hIL-3 pMON13377

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 55 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 60 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser



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Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 289]  
 120 125  
 15 PEPTIDE #B27 Met-Asp-(15-119)hIL-3 pMON13378  
 Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 25 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu [SEQ ID NO.: 290]  
 119  
 40

PEPTIDE #B28 Met-Ala-(15-125)hIL-3 pMON13379

45 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 50 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 55 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
 105 110 115

5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 291]  
 120 125

PEPTIDE #B29 Met-Ala-(15-125)hIL-3 pMON13385

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

15 Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

20 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 292]  
 120 125

35 PEPTIDE #B30 Met-Ala-(15-125)hIL-3 pMON13381

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

40 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40

45 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Trp Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

60 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 293]  
 120 125

## PEPTIDE #B31 Met-Ala-(15-125)hIL-3 pMON13383

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
30 35 40

10 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 294]  
120 125

## 30 PEPTIDE #B32 Met-Ala-(15-125)hIL-3 pMON13384

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

35 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

40 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 295]  
120 125

55

## PEPTIDE #B33 Met-Ala-(15-125)hIL-3 pMON13388

60 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Leu Asn Ala  
 30 35 40  
 5 Glu Asp Val Asp Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 10 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 20 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 296]  
 120 125

## PEPTIDE #B34 Met-Ala-(15-125)hIL-3 pMON13389

25 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 30 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 35 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 40 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 45 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 297]  
 120 125

## 50 PEPTIDE #B35 Met-Ala-(15-125)hIL-3 pMON13391

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 55 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 60 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

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5 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 298]  
 120 125  
 15  
 PEPTIDE #B36 Met-Ala-(15-125)hIL-3 pMON13392  
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 25 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 299]  
 40 120 125

PEPTIDE #B37 Met-Ala-(15-125)hIL-3 pMON13393

45 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 50 Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 300]  
 120 125

PEPTIDE #B38 Met-Ala-(15-125)hIL-3 pMON13394

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 15 30 35 40

Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

20 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 301]  
 120 125

35 PEPTIDE #B39 Met-Ala-(15-125)hIL-3 pMON13395

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25

40 Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

60 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 302]  
 120 125

## PEPTIDE #B40 Met-Ala-(15-125)hIL-3 pMON13396

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
30 35 40

10 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Arg Met Gly  
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 303]  
120 125

## PEPTIDE #B41 Met-Ala-(15-125)hIL-3 pMON13397

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
35 30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
45 50 55

40 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Trp Pro Cys Leu Pro Ser  
75 80 85

45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Arg Met Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 304]  
120 125

## 55 PEPTIDE #B42 Met-Ala-(15-125)hIL-3 pMON13398

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

60 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp

5	Glu	Asp	Val	Ser	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Leu	Pro	Asn
			45					50							55
10	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65							70
15	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80							85
20	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95							100
25	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110							115
30	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 305]						
			120					125							
PEPTIDE #B43 Met-Ala-(15-125)hIL-3 pMON13399															
35	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ala	Ile	His	His	Leu
			15					20							25
40	Lys	Val	Pro	Pro	Arg	Pro	Ser	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Asp
			30					35							40
45	Glu	Asp	Val	Ser	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Leu	Pro	Asn
			45					50							55
50	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65							70
55	Gly	Ile	Glu	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser
			75					80							85
60	Ala	Thr	Ala	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly
			90					95							100
65	Asp	Trp	Gln	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr
			105					110							115
70	Leu	Glu	Gln	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 306]						
			120					125							
PEPTIDE #B44 Met-Ala-(15-119)hIL-3 pMON13404															
75	Met	Ala	Asn	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu
			15					20							25
80	Lys	Arg	Pro	Pro	Ala	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ala
			30					35							40
85	Glu	Asp	Val	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Leu	Pro	Asn
			45					50							55
90	Leu	Glu	Ser	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser
			60					65							70



Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr  
 105 110 115

10 Leu Glu [SEQ ID NO.: 307]  
 119

15 PEPTIDE #B45 Met-Ala-(15-125)hIL-3 pMON13387

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40

Glu Asp Val Asp Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 308]  
 120 125

40

PEPTIDE #B46 Met-Ala-(15-125)hIL-3 pMON13416

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 45 15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40

50 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 309]  
 120 125

PEPTIDE #B47 Met-Ala-(15-125)hIL-3 pMON13417

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40

15 Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55

20 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 310]  
 120 125

35 PEPTIDE #B48 Met-Ala-(15-125)hIL-3 pMON13420

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25

40 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40

Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn  
 45 50 55

45 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70

50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 311]  
 120 125

60

## PEPTIDE #B45 Met-Ala-(15-125)hIL-3 pMON13421

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 10 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.:331]  
 120 125

## PEPTIDE #B50 Met-Ala-(15-125)hIL-3 pMON13432

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp  
 30 35 40  
 35 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 40 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 50 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 312]  
 120 125

## 55 PEPTIDE #B51 Met-Ala-(15-125)hIL-3 pMON13382

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 60 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Trp Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 313]  
 120 125  
 20 PEPTIDE #B52 Met-Asp-(15-125)hIL-3 pMON13476  
 Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 25 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 30 35 40  
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 45 50 55  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 60 65 70  
 35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 45 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 314]  
 120 125  
 PEPTIDE #B53 Met-Ala-(15-125)hIL-3 pMON13446  
 50 Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys Asn  
 -14 -10 -5 15  
 55 Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu Lys Arg Pro  
 20 25 30  
 Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala Glu Asp Val  
 35 40 45  
 60 Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn Leu Glu Ser

				50						55				60	
	Phe	Val	Arg	Ala	Val	Lys	Asn	Leu	Glu	Asn	Ala	Ser	Gly	Ile	Glu
				65						70				75	
5															
	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser	Ala	Thr	Ala
				80						85				90	
10	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly	Asp	Trp	Gln
				95						100				105	
	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr	Leu	Glu	Gln
				110						115				120	
15	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 315]									
				125											

PEPTIDE #B54 Met-Ala-(15-125)hIL-3 pMON13390

20	Met	Ala	Tyr	Pro	Glu	Thr	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Asn
	-14				-10					-5					15
25	Cys	Ser	Ile	Met	Ile	Asp	Glu	Ile	Ile	His	His	Leu	Lys	Arg	Pro
				20						25					30
	Pro	Asn	Pro	Leu	Leu	Asp	Pro	Asn	Asn	Leu	Asn	Ser	Glu	Asp	Met
				35						40				45	
30	Asp	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn	Leu	Leu	Ala
				50						55				60	
	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser	Gly	Ile	Glu
				65						70				75	
35															
	Ala	Ile	Leu	Arg	Asn	Leu	Gln	Pro	Cys	Leu	Pro	Ser	Ala	Thr	Ala
				80						85				90	
	Ala	Pro	Ser	Arg	His	Pro	Ile	Ile	Ile	Lys	Ala	Gly	Asp	Trp	Gln
40				95						100				105	
	Glu	Phe	Arg	Glu	Lys	Leu	Thr	Phe	Tyr	Leu	Val	Thr	Leu	Glu	Gln
				110						115				120	
45	Ala	Gln	Glu	Gln	Gln	[SEQ ID NO.: 316]									
				125											

PEPTIDE #C-2 Met-Ala-(15-125)hIL-3 pMON13400

50	Met	Ala	Asn	Cys	Ser	Ile	Met	Pro	Asp	Glu	Ala	Ile	His	His	Leu
			15					20					25		
	Lys	Ile	Pro	Pro	Asn	Pro	Ser	Leu	Asp	Ser	Ala	Asn	Leu	Asn	Ser
55			30					35					40		
	Glu	Asp	Val	Ser	Ile	Leu	Met	Glu	Arg	Asn	Leu	Arg	Thr	Pro	Asn
			45					50					55		
60	Leu	Leu	Ala	Phe	Val	Arg	Ala	Val	Lys	His	Leu	Glu	Asn	Ala	Ser
			60					65					70		

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 317]  
 120 125  
 15 PEPTIDE #C-3 Met-Ala-(15-125)hIL-3 pMON13402  
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu  
 15 20 25  
 20 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser  
 30 35 40  
 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 25 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100  
 35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 318]  
 120 125  
 40  
 PEPTIDE #C-10 Met-Ala-(15-125)hIL-3 pMON13440  
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu  
 15 20 25  
 45 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser  
 30 35 40  
 50 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70  
 55 Gly Ile Glu Pro Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85  
 60 Ala Thr Ala Ala Pro Ser Arg Thr Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 319]  
 120 125

PEPTIDE #C-11 Met-Ala-(15-125)hIL-3 pMON13451

10 Met Ala Asn Cys Ser Ile Ile Leu Asp Glu Ala Ile His His Leu  
 15 15 20 25

15 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser  
 30 35 40

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55

20 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70

Gly Ile Glu Pro Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

25 Ala Thr Ala Ala Pro Ser Arg Thr Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 320]  
 120 125

35 PEPTIDE #C-4 Met-Ala-(15-125)hIL-3 pMON13403

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25

40 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40

45 Glu Asp Met Asp Ile Leu Met Asp Ser Asn Leu Arg Thr Pro Asn  
 45 50 55

Leu Leu Ala Phe Pro His Ala Ser Lys Gln Leu Glu Asn Ala Ser  
 60 65 70

50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115

60 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 321]  
 120 125

10



PEPTIDE #C-1 Met-Ala-(15-125)hIL-3 pMON13413

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

20 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Ser  
105 110 115

25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 324]  
120 125

PEPTIDE #C-8 Met-Ala-(15-125)hIL-3 pMON13419

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
15 20 25

35 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
30 35 40

Glu Asp Met Asp Ile Leu Met Asp Ser Asn Leu Leu Thr Pro Asn  
45 50 55

40 Leu Leu Ala Phe Pro His Ala Ser Lys Gln Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

50 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Ser  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 325]  
120 125

55

PEPTIDE #C-9 Met-Ala-(15-125)hIL-3 pMON13418

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40  
 10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70  
 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr  
 75 80 85  
 20 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly  
 90 95 100  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 326]  
 120 125

30 PEPTIDE #C-9 Met-Ala-(15-125)hIL-3 pMON13428

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 15 20 25  
 35 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 30 35 40  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70  
 45 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr  
 75 80 85  
 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly  
 90 95 100  
 50 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr  
 105 110 115  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 327]  
 120 125  
 55

PEPTIDE #C- Met-Ala-(15-125)hIL-3 pMON13459

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu  
15 20 25

Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser  
30 35 40

10 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr  
75 80 85

20 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly  
90 95 100

25 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr  
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 328]  
120 125

30

PEPTIDE #C-13 Met-Ala-(15-125)hIL-3 pMON13467

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu  
15 20 25

Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser  
30 35 40

40 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
60 65 70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
90 95 100

Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Ser  
105 110 115

55 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 329]  
120 125

PEPTIDE #C Met-Ala-(15-125)hIL-3 pMON1349

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu  
 15 20 25  
 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser  
 30 35 40  
 10 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 45 50 55  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 60 65 70  
 15 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr  
 75 80 85  
 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly  
 90 95 100  
 20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 105 110 115  
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 330]  
 120 125

TABLE 4  
DNA SEQUENCES

30 pMON13287  
 Met-Ala-(15-125) IL-3  
 35 DNA sequence #1  
 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
 40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
 45 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
 GAACAACAG [SEQ ID NO:97]  
 50 pMON13290  
 Met-Ala-(15-125) IL-3  
 DNA sequence #2  
 55 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
 60 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:98]

5

pMON13313

10 Met-Ala-(15-125) IL-3

DNA sequence #3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG

15 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT

CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG

20 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCAG

GAACAACAG [SEQ ID NO:99]

25

pMON13288

Met-Ala-(15-125) IL-3

30 DNA sequence #4

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACCTCCA

35 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG

40 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG

GAACAACAG [SEQ ID NO:100]

45 pMON13312

Met-Ala-(15-125) IL-3

DNA sequence #5

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG

50 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACCTCCA

AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT

CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG

55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG

GAACAACAG [SEQ ID NO:101]

60

pMON13294

Met-Ala-(15-125) IL-3

5 DNA sequence #6  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
10 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCAG  
15 GAACAACAG [SEQ ID NO:102]

pMONM13289

20 Met-Ala-(15-125) IL-3

DNA sequence #7  
25 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG  
CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:103]

35

pMON13292

40 Met-Ala-(15-125) IL-3

DNA sequence #8  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG  
CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
45 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCTTGAGCAAGCGCAG  
50 GAACAACAG [SEQ ID NO:104]

55 pMON13295

Met-Ala-(15-125) IL-3

DNA sequence #9  
60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG

CTGGACAGTAAACCTCAATTCCGAAGACATGGATATCCTGATGACGAAACCTTCGACTTCCA  
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGGTATTGAGGCAATTCTT  
5 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
GCAGGTGACTGGCAAGAATTCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCAG  
GAACAACAG [SEQ ID NO:105]

10

pMON13344

(15-125) IL-3

15

DNA sequence #10

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG

20

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAAAATAACCTTCGTCGTCCA

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCTT

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

25

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

GCTCAACAG [SEQ ID NO:106]

30

pMON13345

(15-125) IL-3

DNA sequence #11

35

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAAAATAACCTTCGTCGTCCA

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCTT

40

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

45

GCTCAACAG [SEQ ID NO:107]

pMON13346

50

(15-125) IL-3

DNA sequence #12

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG

55

CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAAAATAACCTTCGTCGTCCA

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCTT

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

60

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

GCTCAACAG [SEQ ID NO:108]

5

**PMON13347**

(15-125) IL-3

10 **DNA sequence #13**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGACTTCCA

15 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT

AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

20

GCTCAACAG [SEQ ID NO:109]

**PMON13348**

25

(15-125) IL-3

**DNA sequence #14**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

30

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGACTTCCA

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT

35 AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

40

GCTCAACAG [SEQ ID NO:110]

**PMON13349**

(15-125) IL-3

45

**DNA sequence #15**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGAACTCCA

50

AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT

AAAAATCTCCTGCCATGTCTGCCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

55 GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

GCTCAACAG [SEQ ID NO:111]



pMON13350

(15-125) IL-3

5 DNA sequence #16

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGTCCTCA

10 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG

15 GCAGGTGACTGGCAAGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGGAGAACGCGCAG

GCTCAACAG [SEQ ID NO:112]

pMON13355

20

(15-125) IL-3

DNA sequence #17

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

25

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGTCCTCA

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT

30 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG

GCAGGTGACTGGCAAGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGGAGAACGCGCAG

35 GCTCAACAG [SEQ ID NO:113]

pMON13352

40

(15-125) IL-3

DNA sequence #18

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

45 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGTCCTCA

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCTT

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG

50 GACGGTGACTGGAATGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG

GAACAACAG [SEQ ID NO:114]

55 pMON13354

(15-125) IL-3

DNA sequence #19

60 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACCTCAATGGTGAAGACCAAGATATCCTGATGATAAACCTTCGTCGTCCA  
AACCTCGAGGCATTCAACCGTGTCTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCTT  
5 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
GACGGTGACTGGAATGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCAG  
GAACAACAG [SEQ ID NO:115]  
10

PMON13363

(15-125) IL-3 SECRETED

DNA sequence #20

AACCTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT  
AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
25 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG  
GCTCAACAG [SEQ ID NO:116]

PMON13364

(15-125) IL-3 SECRETED

DNA sequence #21

AACCTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT  
40 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG  
45 GCTCAACAG [SEQ ID NO:117]

PMON13365

(15-125) IL-3 SECRETED

DNA sequence #22

AACCTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG  
55 CTGGACAGTAACAACCTCAATTCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT  
AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
60 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG

GCTCAACAG [SEQ ID NO:118]

5 pMON13360

(15-125) IL-3 SECRETED

DNA sequence #23

10 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG  
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCCA  
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT  
15 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
20 GAACAACAG [SEQ ID NO:119]

pMON13361

25 (15-125) IL-3 SECRETED

DNA sequence #24

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG  
30 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCCA  
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
35 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:120]

40

pMON13362

(15-125) IL-3 SECRETED

45 DNA sequence #25

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG  
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCCA  
50 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCAG  
55 GAACAACAG [SEQ ID NO:121]

pMON13301

(15-125) IL-3 INTRACELLULAR

5 DNA sequence #26  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
10 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT  
AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
15 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG  
GCTCAACAG [SEQ ID NO:122]

pMON13302

20 (15-125) IL-3 INTRACELLULAR

DNA sequence #27  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
25 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT  
30 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG  
35 GCTCAACAG [SEQ ID NO:123]

pMON13303

40 (15-125) IL-3 INTRACELLULAR

DNA sequence #28  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG  
CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
45 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCTT  
AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAG  
50 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCAG  
GCTCAACAG [SEQ ID NO:124]

55 pMON13298

(15-125) IL-3 INTRACELLULAR

DNA sequence #29  
60 ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACCTCAATGGTGAAGACCAAGATATCCTGATGATAAACCTTCGTCGTCCA  
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT  
5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:125]  
10 pMON13299  
(15-125) IL-3 INTRACELLULAR  
15 DNA sequence #30  
ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG  
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCCA  
20 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
25 GAACAACAG [SEQ ID NO:126]  
pMON13300  
30 Met-Ala-(15-125) IL-3 INTRACELLULAR  
DNA sequence #31  
35 ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG  
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCCA  
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCTT  
40 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCAG  
GAACAACAG [SEQ ID NO:127]  
45 DNA sequence #32  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
50 AACCTGCTCGCATTCTGAAGGGCTGTCAAGCACTTAGAAAACGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO: 160]  
60 DNA sequence #33  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG

CTGGACCCGAACAACCTCAATTCTGAAGACATGGACATTTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAACGCATCAGGTATTGAGGCAATTCTT  
5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
10 GAACAACAG [SEQ ID NO: 161]  
DNA sequence #B1 pMON13406 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
15 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
20 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
25 GAACAACAG [SEQ ID NO.: 332]  
DNA sequence #B2 pMON13414 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
30 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
35 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
40 GAACAACAG [SEQ ID NO.: 333]  
DNA sequence #B3 pMON13407 Met-Ala-(15-125) IL-3  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
45 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
55 GAACAACAG [SEQ ID NO.: 334]  
DNA sequence #B4 pMON13405 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
60 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCCA

AACCTGCTCGCATTGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
5 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 335]

10 DNA sequence #B5 pMON13415 Met-Ala-(15-125) IL-3  
ATGGCAAAGTCTCTATAATGATCCATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
15 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
20 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 336]

25 DNA sequence #B6 pMON13408 Met-Ala-(15-125) IL-3  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
30 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATCGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
35 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 337]

40 DNA sequence #B7 pMON13409 Met-Ala-(15-125) IL-3  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
45 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGCTGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
50 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 338]

55 DNA sequence #B8 pMON13410 Met-Ala-(15-125) IL-3  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
60 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGGACGAAACCTTCGAACTCCA

AACCTGCTCGC[REDACTED]GTAAAGGGCTGTCAAGCACTTAGAAAATGCA[REDACTED]GTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
5 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 339]

10 DNA sequence #B9 pMON13422 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATCGAACGAAACCTTCGAACCTCCA  
15 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
20 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 340]

25 DNA sequence #B10 pMON13423 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATCGAACGAAACCTTCGAACCTCCA  
30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
35 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 341]

40 DNA sequence #B11 pMON13424 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGCTGGAACGAAACCTTCGAACCTCCA  
45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
50 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 342]

55 DNA sequence #B12 pMON13425 Met-Ala-(15-125) IL-3  
ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGCTGGAACGAAACCTTCGAACCTCCA  
60 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT



CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
5 GAACAACAG [SEQ ID NO.: 343]

DNA sequence #B13 pMON13426 Met-Ala-(15-125) IL-3  
10 ATGGCAAAGTCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGGACGAACGAAACCTTCGAACTCCA  
15 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
20 GAACAACAG [SEQ ID NO.: 344]

DNA sequence #B14 pMON13429 Met-Ala-(15-125) IL-3  
25 ATGGCAAAGTCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTG  
CTGGACCCGAACAACCTCAATTCTGAAGACGTTATATCCTGGACGAACGAAACCTTCGAACTCCA  
30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
35 GAACAACAG [SEQ ID NO.: 345]

DNA sequence #B15 pMONM13368 Met-Ala-(15-125) IL-3  
40 ATGGCTAAAGTCTCTATTATGATCGATGAAGCAATACATCACTTAAAGGTTCCACCTGCACCTTG  
CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
50 GAACAACAG [SEQ ID NO.: 346]

DNA sequence #B16 pMONM13380 Met-Ala-(15-125) IL-3  
55 ATGGCTAAAGTCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTTCCACCTGCACCTTG  
CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
60 AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT

CGTAATCTCCAAATGTCTGCCCTCTGCCACGGCCGCACCCTCTATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCAG  
5 GAACAACAG [SEQ ID NO.: 347]

DNA sequence #B17 pMON13475 Met-Ala-(15-125) IL-3

10 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
15 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
20 GAACAACAG [SEQ ID NO.: 348]

DNA sequence #B18 pMON13366 Met-Ala-(15-125) IL-3

25 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATAACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
35 GAACAACAG [SEQ ID NO.: 349]

DNA sequence #B19 pMON13367 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
50 GAACAACAG [SEQ ID NO.: 350]

DNA sequence #B20 pMON13369 Met-Ala-(15-125) IL-3 42D, 46S, 50D

55 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
60 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG

GCAGGTGACTGGAAGAATTCCGGGAAAAACTGACGTTCTATCTGACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.:351]

5

DNA sequence #B21 pMON13370 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
10 CTGGACCCGAACAACCTCAATGCTGAAGACATGTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
15 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 352]

20

DNA sequence #B22 pMON13373 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
25 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
30 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 353]

35

DNA sequence #B23 pMON13374 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
40 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
45 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 354]

50

DNA sequence #B24 pMON13375 Met-Ala-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
55 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
60

GCAGGTGACTGAGAAATTCGGGAAAAACTGACGTTCTATCTGACCCTTGAG [SEQ ID NO.: 355]

5 DNA sequence #B25 pMON13376 Met-Asp-(15-119) IL-3  
ATGGATAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
10 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
15 GCAGGTGACTGGCAAGAATTCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID NO.: 356]

DNA sequence #B26 pMON13377 Met-Ala-(15-119) IL-3  
20 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGACGAAGACGTCCTATTCTGATGGACCGAAACCTTCGACTTCCA  
25 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID NO.: 357]  
30

DNA sequence #B27 pMON13378 Met-Asp-(15-119) IL-3  
35 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGGTATTGAGGCAATTCTT  
40 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAG [SEQ ID NO.: 358]  
45

DNA sequence #B28 pMON13379 Met-Ala-(15-125) IL-3  
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
50 CTGGACCCGAACAACCTCAATGCTGAAGACGTTTCTATCCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 359]  
60

DNA sequence #B29 pMON13385 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTACCACCTCGCCCTTCC  
5 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
10 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 360]

15 DNA sequence #B30 pMON13381 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
20 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCTGGCCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 361]

30 DNA sequence #B31 pMON13383 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTTG  
35 CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTCAATTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 362]

45 DNA sequence #B32 pMON13384 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
50 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTCAATTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 363]

60

DNA sequence #B33 pMON13388 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
5 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGACCGAAACCTTCGACTTAGC  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
10 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 364]

15 DNA sequence #B34 pMON13389 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
20 CTGGACCCGAACAACCTCAATGACGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 365]

30 DNA sequence #B35 pMON13391 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTCC  
35 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 366]

45 DNA sequence #B36 pMON13392 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
50 CTGGACCCGAACAACCTCAATGACGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 367]

60

DNA sequence #B37 pMON13393 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTCC  
5 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
10 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 368]

15 DNA sequence #B38 pMON13394 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
20 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 369]

30 DNA sequence #B39 pMON13395 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTACCACCTCGCCCTTCC  
35 CTGGACCCGAACAACCTCAATGACGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 370]

45 DNA sequence #B40 pMON13396 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
50 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCCGT  
55 ATGGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 371]

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DNA sequence #B41 pMON13397 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
5 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCTGGCCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCCGT  
10 ATGGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 372]

15 DNA sequence #B42 pMON13398 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
20 CTGGACCCGAACAACCTCAATGACGAAGACGTCCTCTATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 373]

30 DNA sequence #B43 pMON13399 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTACCACCTCGCCCTTCC  
35 CTGGACCCGAACAACCTCAATGACGAAGACGTCCTCTATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 374]

45 DNA sequence #B44 pMON13404 Met-Ala-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
50 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID  
NO.: 375]



DNA sequence #B45 pMON13387 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
5 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGACCGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
10 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 376]

15 DNA sequence #B46 pMON13416 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
20 CTGGACCCGAACAACCTCAATGACGAAGACGTCGATTCTCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 377]

30 DNA sequence #B47 pMON13287 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
35 CTGGACCCGAACAACCTCAATGACGAAGACGTCATGTCTCTGATGGAACGAAACCTTCGACTTCCA  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 378]

45 DNA sequence #B48 pMON13420 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGACCTTCC  
50 CTGGACCCGAACAACCTCAATGACGAAGACGTCCTATCCTGATGGACCGAAACCTTCACTTAGC  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAAAGTACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 379]

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DNA sequence #B49 pMON13421 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTCC  
5 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGACCGAAACCTTCGACTTAGC  
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
10 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 380]

15 DNA sequence #B50 pMON13432 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTCC  
CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGACCGAAACCTTCGACTTCCA  
20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 381]

30 DNA sequence #B51 pMON13382 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
35 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGTGGACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 382]

45 DNA sequence #B52 pMON13476 Met-Asp-(15-125) IL-3

ATGGATAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTTG  
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCA  
50 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO.: 383]

pMON13400

Met-Ala- (15-125) IL-3

5 DNA sequence #C2

ATGGCTAACTGCTCTATAATGCCAGATGAAGCAATACATCACTTAAAGATACCACCTAACCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
10 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
15 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:384]

20 pMON13402

Met-Ala- (15-125) IL-3

25 DNA sequence #C3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
35 GAACAACAG [SEQ ID NO:385]

40 pMON13440

Met-Ala- (15-125) IL-3

DNA sequence #C10

45 ATGGCTAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGATACCACCTAACCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
55 GAACAACAG [SEQ ID NO:386]

pMON13451

Met-Ala-(15-125) IL-3

5 DNA sequence #C11

ATGGCTAACTGCTCTATAATACTCGATGAAGCAATACATCACTTAAAGATACCACCTAACCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
10 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
15 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:387]

20 pMON13403

Met-Ala-(15-125) IL-3

25 DNA sequence #C4

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGACTCCAACCTTCGAACTCCA  
30 AACCTGCTCGCATTCCCACATGCTGTCAAGCAATTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
35 GAACAACAG [SEQ ID NO:388]

40 pMON13419

Met-Ala-(15-125) IL-3

DNA sequence #C8

45 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGACTCCAACCTTCGAACTCCA  
AACCTGCTCGCATTCCCACATGCTTCTAAGCAATTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
55 GAACAACAG [SEQ ID NO:389]

pMON13411

Met-Ala-(15-125) IL-3

5 DNA sequence #C5

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
10 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
15 GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:390]

20 pMON13412

Met-Ala-(15-118) IL-3

25 DNA sequence #C6

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTTAATA  
35 [SEQ ID NO:391]

pMON13413

40 Met-Ala-(15-125) IL-3

DNA sequence #C7

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG  
45 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTTCTCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:392]  
55

pMON13418

Met-Ala-(15-125) IL-3

5 DNA sequence #C1

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTTG  
CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
10 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCTT  
TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAAG  
15 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:393]

20 pMON13428

Met-Ala-(15-125) IL-3

25 DNA sequence #C9

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTTG  
CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCCA  
30 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCTT  
TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAAG  
GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCAG  
35 GAACAACAG [SEQ ID NO:394]

40 pMON13459

Met-Ala-(15-125) IL-3

45 DNA sequence #C12

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCTT  
50 TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAAG  
GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCAG  
55 GAACAACAG [SEQ ID NO:395]

pMON13467

Met-Ala-(15-125) IL-3

5 DNA sequence #C13

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
10 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTT  
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAG  
15 GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTTCTCTTGAGCAAGCGCAG  
GAACAACAG [SEQ ID NO:396]

20 pMON13492

Met-Ala-(15-125) IL-3

25 DNA sequence #C14

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCCTAGC  
CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCCA  
30 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCTT  
TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCTCCCTCTCGAACACCAATCAGATCAAG  
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAG  
35 GAACAACAG [SEQ ID NO:397]

40 pMON13446

Met-Ala-Tyr-Pro-Glu-Thr-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-Ala (15-125) IL-3

45 DNA sequence #B53

ATGGCATATCCAGAACTGATTACAAGGACGACGATGACAAGGCTAACTGCTCTATAATGATCGAT  
GAAATTATACATCACTTAAAGAGACCACCTGCACCTTTGCTGGACCCGAACAACCTCAATGCTGAA  
50 GACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCAAACCTGGAGAGCTTCGTAAGGGCTGTC  
AAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTTCGTAATCTCCAACCATGTCTGCCCTCT  
GCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAGGCAGGTGACTGGCAAGAATTCCGGGAA  
55 AACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAGGAACAACAG [SEQ ID NO:404]

pMON13390

Met-Ala-Tyr-Pro-Glu-Thr-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-Ala (15-125) IL-3

5

DNA sequence #B54

ATGGCATATCCAGAACTGATTACAAGGACGACGATGACAAGGCTAACTGCTCTATAATGATCGAT  
 10 GAAATTATACATCACTTAAAGAGACCACCTAACCCCTTGCTGGACCCGAACAACCTCAATTCCGAA  
 GACATGGATATCCTGATGGAACGAAACCTTCGAACTCCAAACCTGCTCGCATTTCGTAAGGGCTGTC  
 15 AAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTTCGTAATCTCCAACCATGTCTGCCCTCT  
 GCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAGGCAGGTGACTGGCAAGAATTCCGGGAA  
 AACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAGGAACAACAG [SEQ ID NO:405]

20

Polypeptides corresponding to [SEQ ID NO. 129] comprising (1-133)hIL-3 containing four or more amino acid substitutions can be made using the procedures described above and in the following examples by starting with the appropriate oligonucleotides and then constructing the DNA encoding the polypeptide and expressing it in an appropriate host cell. In a similar manner polypeptides which correspond to [SEQ ID NO. 130] and contain four or more amino acid substitutions and wherein from 1 to 14 amino acids have been sequentially deleted from the N-terminus, or from 1 to 15 amino acids have been deleted from the C-terminus or deletions of amino acids have been made from both the N-terminus and the C-terminus can also be made by following the procedures described above and in the following examples, beginning with the appropriate starting materials.

Further details known to those skilled in the art may be found in T. Maniatis, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory (1982) and references cited therein, incorporated herein by reference; and in J. Sambrook, et al., Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory (1989) and references cited therein, incorporated herein by reference.



The following examples will illustrate the invention in greater detail although it will be understood that the invention is not limited to these specific examples.

Amino acids are shown herein by standard one letter or three letter abbreviations as follows:

	Abbreviated Designation	Amino Acid
10	A Ala	Alanine
	C Cys	Cysteine
	D Asp	Aspartic acid
	E Glu	Glutamic acid
15	F Phe	Phenylalanine
	Abbreviated Designation	Amino Acid
	G Gly	Glycine
20	H His	Histidine
	I Ile	Isoleucine
	K Lys	Lysine
	L Leu	Leucine
	M Met	Methionine
25	N Asn	Asparagine
	P Pro	Proline
	Q Gln	Glutamine
	R Arg	Arginine
	S Ser	Serine
30	T Thr	Threonine
	V Val	Valine
	W Trp	Tryptophan
	Y Tyr	Tyrosine

Various other examples will be apparent to the person skilled in the art after reading the present disclosure without departing from the spirit and scope of the invention. It is intended that all such other examples be included within the scope of the appended claims.

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30

#### EXAMPLE 1

Construction of pMON 5846 (Fig. 4) which encodes [Met-(1-133)hIL-3 (Arg129)]

35

A plasmid containing the gene for the cDNA of hIL-3 cloned into pUC18 on an EcoRI to HindIII fragment was

obtained from British Biotechnology Limited (Cambridge, England). This plasmid was designated pPO518. The purified plasmid DNA was cleaved by the restriction endonucleases NheI and BamHI. Approximately 0.5  
 5 micrograms of cleaved plasmid DNA was ligated to 1.0 picomoles of a pair of annealed oligonucleotides with the following sequence:

5'-CTAGCGATCTTTTAATAAGCTTG-3' [SEQ ID NO: 1]  
 10 3'-GCTAGAAAATTATTCTGAACCTAG-5' [SEQ ID NO: 2]

The ligation mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked, and plasmid DNA was purified and subjected to  
 15 restriction enzyme analysis. An isolate was identified in which the above oligonucleotide sequence had replaced the portion of the gene that encodes the extreme C-terminus. Within the new sequence was a new stop codon, TAA, and a recognition site for the enzyme HindIII. The  
 20 new plasmid was designated pMON5846.

## EXAMPLE 2

### (a) Construction of expression vector plasmid pMON2341

25 The plasmid pMON2341 was used to supply the particular replicon and expression elements used for construction of many of the plasmids used to produce hIL-3 and hIL-3 muteins in *E. coli*. These expression elements are described in the materials and methods  
 30 section. pMON2341 is derived from pMON5515 (Olins et al., 1988) and from pMON2429. pMON2429 consists of the phage mp18 (Yanisch-Perron et al., 1985) with a BclI fragment carrying the chloramphenicol acetyl transferase (cat) gene from pBR328 (Covarrubias et al., 1981)  
 35 inserted into the BamHI site. The cat gene in pMON2429 has been altered from that in pBR328 by site directed mutagenesis (Kunkel, 1985). The recognition sites for

NcoI and EcoRI which occur in the native gene were altered so that these two restriction enzymes no longer recognize these sites. The changes did not alter the protein specified by the gene. Also, an NcoI site was  
5 introduced at the N-terminus of the coding sequence so that it overlaps the codon for initiator methionine.

The steps involved in construction of pMON2341 are listed below:

(1) The DNAs of pMON5515 and pMON2429 were treated  
10 with NcoI and HindIII. The fragments were ligated and used to transform competent *E. coli* to ampicillin resistance. From these colonies, some were identified that were chloramphenicol resistant. From one of these colonies, plasmid DNA was isolated in which the rat  
15 atriopetigen gene of pMON5515 had been replaced by the NcoI to HindIII fragment containing the cat gene from pMON2429. This fragment contains the recognition sites for several restriction enzymes in the portion derived from the multilinker region of mp18. The new plasmid was  
20 designated pMON2412.

(2) pMON2412 was treated with the enzyme ClaI which cleaves at one location in the pBR327 derived portion of the DNA. The protruding ends were rendered blunt by treatment with Klenow in the presence of nucleotide  
25 precursors. This DNA was mixed with an isolated 514 bp RsaI fragment derived from pEMBL8 (Dente et al., 1983). This RsaI fragment contains the origin of replication of phage f1. This ligation mixture was used to transform competent *E. coli* cells to ampicillin resistance. Among  
30 the plasmid DNAs isolated from these cells was pMON5578. This plasmid has the structure of pMON2412 with the f1 origin region inserted into the ClaI site. This is illustrated in the Figures and in Olins and Rangwala (1990).

35 (3) The DNA of pMON5578 was treated with restriction enzymes HindIII and MstII. The DNA was then treated with Klenow enzyme in the presence of nucleotide precursors to

render the ends blunt. This treated DNA was ligated and used to transform competent *E. coli* to ampicillin resistance. From the ampicillin resistant colonies, one plasmid was recovered from which the portion between  
 5 HindIII and MstII was absent. This deletion resulted in the removal of sequences from the plasmid which are recognized by a number of restriction endonuclease sites. The new plasmid was designated pMON5582.

(4) The DNA of pMON5582 was treated with SstII and  
 10 BclII and ligated in the presence of annealed oligonucleotides with the sequences shown below.

5'- GGCAACAATTTCTACAAAACACTTGATACTGTATGAGCAT-  
 3'-CGCCGTTGTTAAAGATGTTTTGTGAACTATGACATACTCGTA-

15

ACAGTATAATTGCTTCAACAGAACAGATC-3' [SEQ ID NO:3]  
 TGTCATATTAACGAAGTTGTCTTGT-5' [SEQ ID NO:4]

This sequence encodes the essential elements of the  
 20 *recA* promoter of *E. coli* including the transcription start site and the *lexA* repressor binding site (the operator) (Sancar et al., 1980). The plasmid recovered from the ligation mixes contained this *recA* promoter in place of the one in pMON5582 (and in pMON5515). The  
 25 functionality of the *recA* promoter was illustrated by Olins and Rangwala (1990). The new plasmid was designated pMON5594.

(5) To eliminate the single EcoRI site in pMON5594, the DNA was treated with EcoRI, then with Klenow in the  
 30 presence of nucleotide precursors to render the ends blunt and then the DNA was ligated. From this ligation mix a plasmid was recovered whose DNA was not cleaved with EcoRI. This plasmid was designated pMON5630.

(6) To alter the single recognition site for PstI, plasmid pMON5630 was subjected to site directed  
 35 mutagenesis (Kunkel, 1985). The oligonucleotide used in this procedure has the sequence shown below.

5'-CCATTGCTGCCGGCATCGTGGTC-3' [SEQ ID NO:5]

The result of the procedure was to construct pMON2341 which differs from pMON5630 in that the PstI site in the beta-lactamase gene was altered so that PstI no longer recognizes the site. The single nucleotide change does not alter the amino acid sequence of the beta-lactamase protein.

(b) Construction of pMON5847 (Fig. 5) which encodes [Met-(1-133)hIL-3(Arg129)]

Plasmid pMON2341 was used to supply the replicon, promotor, ribosome binding site, transcription terminator and antibiotic resistance marker for the plasmids used to produce hIL-3 in *E. coli* from cDNA derived hIL-3 genes.

Plasmid pMON2341 was treated with restriction enzymes NcoI and HindIII. The restriction fragment containing the replication origin was purified. The DNA of plasmid pMON5846 was treated with NcoI and HindIII. The restriction fragment containing the hIL-3 gene was gel purified. These purified restriction fragments were mixed and ligated. The ligation mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked, and plasmid DNA was purified and analyzed using restriction enzymes. pMON5847 was identified as a plasmid with the replicon of pMON2341 and the hIL-3 gene in place of the chloramphenicol acetyl transferase gene. JM101 cells harboring this plasmid were cultured in M9 medium and treated with nalidixic acid as described above. Samples of the culture were examined for protein content. It was found that this hIL-3 mutein was produced at about 6% of total cell protein as measured on Coomassie stained polyacrylamide gels.

## EXAMPLE 3

Construction of pMON5854 (Fig. 7) which encodes [Met-(1-133)hIL-3(Arg129)]

To increase the accumulation of hIL-3 in *E. coli*,  
 5 the coding sequence of the amino terminal portion of the  
 protein was altered to more closely reflect the codon  
 bias found in *E. coli* genes that produce high levels of  
 proteins (Gouy and Gautier, 1982). To change the coding  
 sequence for the amino terminal portion of the gene, a  
 10 pair of synthetic oligonucleotides were inserted between  
 the NcoI and HpaI sites within the coding sequence.  
 About 0.5 micrograms of DNA of the plasmid pMON5847  
 (Example 2) was treated with NcoI and HpaI. This DNA was  
 mixed with an annealed pair of oligonucleotides with the  
 15 following sequence:

5'-CATGGCTCCAATGACTCAGACTACTTCTCTTAAGACT-  
 3'-CGAGGTTACTGAGTCTGATGAAGAGAATTCTGA-

20 TCTTGGGTT-3' [SEQ ID NO:6]  
 AGAACCCAA-5' [SEQ ID NO:7]

The fragments were ligated. The ligation mixture  
 was used to transform competent JM101 to ampicillin  
 25 resistance. Colonies were picked into broth. From the  
 cultures plasmid DNA was made and examined for the  
 presence of a DdeI site (CTNAG) which occurs in the  
 synthetic sequence but not between the NcoI and HpaI  
 sites in the sequence of pMON5847. The new recombinant  
 30 plasmid was designated pMON5854. The nucleotide sequence  
 of the DNA in the coding sequence of the amino terminal  
 portion of the hIL-3 gene in pMON5854 was determined by  
 DNA sequencing and found to be the same as that of the  
 synthetic oligonucleotide used in ligation. Cultures of  
 35 JM101 cells harboring this plasmid were grown and treated  
 with nalidixic acid to induce production of the hIL-3  
 mutant protein. Analysis of the proteins on Coomassie

gels showed that the accumulation of hIL-3 mutein was about 25% of total cell protein in cultures harboring pMON5854, significantly higher than it was in cultures harboring pMON5847.

5

#### EXAMPLE 4

Construction of pMON5887 (Fig. 12) which encodes [Met-(1-125)hIL-3]

The plasmid DNA of pMON5854 (Example 3) was treated with EcoRI and HindIII and the larger fragment gel was purified. About 0.5 microgram of this DNA was ligated to 1 picomole of an annealed pair of oligonucleotides which encode amino acids 107 through 125 of hIL-3. The sequences of these oligonucleotides are shown below.

15

EcoRI to HindIII

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAAA-

3'-GGCAGCATTGTGACTGGAAGATAGACTTTT-

20 CCTTGGAGAACGCGCAGGCTCAACAGTAATA-3' [SEQ ID NO:8]  
GGAACCTCTTGCGCGTCCGAGTTGTCATTATTCGA-5' [SEQ ID NO:9]

After ligation, the DNA was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked into broth and plasmid DNA was isolated from each culture. Restriction analysis of the plasmid DNA showed the presence of an EcoRI to HindIII fragment smaller than that of pMON5854. The nucleotide sequence of the portion of the coding sequence between the EcoRI and HindIII sites was determined to confirm the accuracy of the replaced sequence. The new plasmid was designated pMON5887 encoding Met-(1-125)hIL-3 which has the following amino acid sequence:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser  
35 Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn

Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala  
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp  
 5 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys  
 Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:10]

#### EXAMPLE 5

##### 10 Construction of pMON5967 which encodes [Met-Ala-(15-125)hIL-3]

Plasmid DNA of pMON5887 isolated from *E. coli* GM48 (dam-) was cleaved with NcoI and ClaI and ligated to 1 picomole of an annealed pair of oligonucleotides,  
 15 encoding amino acids [Met Ala (15-20)hIL-3]. The sequence of these oligonucleotides is shown below.  
 5'-CATGGCTAACTGCTCTAACATGAT-3' [SEQ ID NO:11]

3'-CGATTGACGAGATTGTACTAGC-5' [SEQ ID NO:12]

The resulting ligation mix was used to transform  
 20 competent *E. coli* JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be smaller than that of pMON5887 by restriction analysis using NcoI and NsiI. The nucleotide sequence of the  
 25 region between NcoI and ClaI was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5967 and cells containing it were induced for protein production. Sonicated cell pellets and supernatants were used for protein  
 30 purification and bio-assay.

#### EXAMPLE 6

##### 35 Construction of pMON5978 which encodes [Met-Ala-(15-125)hIL-3]

Plasmid DNA of pMON5967 isolated from *E. coli* GM48(dam-) was cleaved with ClaI and NsiI and ligated to



1 picomole of an annealed assembly of six  
 oligonucleotides encoding hIL-3 amino acids 20-70  
 (FIG. 2). This synthetic fragment encodes three unique  
 restriction sites, EcoRV, XhoI and PstI. The sequence of  
 5 these oligonucleotides is shown in Figure 2.

The resulting ligation mix was used to transform  
 competent *E. coli* JM101 cells to ampicillin resistant  
 colonies. Plasmid DNA was isolated and screened with  
 XbaI and EcoRV for the presence of the new restriction  
 10 site EcoRV. The DNA sequence of the region between ClaI  
 and NsiI was determined and found to be the same as that  
 of the synthetic oligonucleotides. The new plasmid was  
 designated pMON5978, and cells containing it were induced  
 for protein production. Sonicated cell pellets and  
 15 supernatants were used for protein purification and bio-  
 assay.

Plasmid pMON5978 encodes [Met-Ala-(15-125)hIL-3]  
 which has the following amino acid sequence:

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
 20 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala  
 25 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp  
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys  
 Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:13]

### 30 EXAMPLE 7

#### Construction of pMON13356

Plasmid pMON5988 DNA was digested with restriction  
 enzymes NcoI and EcoRV, and the resulting 4190 base pair  
 NcoI,EcoRV fragment contains the following genetic  
 35 elements: beta-lactamase gene (AMP), pBR327 origin of  
 replication, phage f1 origin of replication as the  
 transcription terminator, pAraBAD promoter, g10L ribosome

binding site, lamB secretion leader and the bases  
encoding amino acids 47-125 of (15-125)hIL-3. The 4190  
base pair NcoI,EcoRV restriction fragment from pMON5988  
was ligated to the following annealed complementary  
5 oligonucleotides from Table (2).

**Oligo #13** [SEQ ID NO:27]

**Oligo #14** [SEQ ID NO:28]

The ligation reaction mixture was used to transform  
10 E. coli K-12 strain JM101 and transformant bacteria were  
selected on ampicillin-containing plates. Plasmid DNA  
was isolated from a colony grown in LB broth and the size  
of the inserted fragment was determined by restriction  
analysis employing restriction enzymes NcoI and HindIII  
15 in double digest. In the resulting plasmid the 99 bases  
between the NcoI and EcoRV restriction sites in the (15-  
125) hIL-3 gene are replaced with 22 bases from the above  
mentioned oligonucleotides. This linker also contains a  
NdeI recognition sequence.

20

#### EXAMPLE 8

##### Construction of pMON13344

Plasmid pMON13356 DNA was digested with restriction  
25 enzymes NcoI and EcoRV, and the resulting 4190 base pair  
NcoI,EcoRV fragment contains the following genetic  
elements: beta-lactamase gene (AMP), pBR327 origin of  
replication, phage fl origin of replication as the  
transcription terminator, pAraBAD promoter, g10L ribosome  
30 binding site, lamB secretion leader and the bases  
encoding amino acids 47-125 of (15-125)hIL-3. The second  
DNA fragment was generated by synthetic gene assembly  
using the following complementary oligonucleotide pairs  
that have overlapping ends:

35

Oligo #1 [SEQ ID NO:15]

Oligo #2 [SEQ ID NO:16]

Oligo #3 [SEQ ID NO:17]

5 Oligo #4 [SEQ ID NO:18]

Oligo #9 [SEQ ID NO:23]

Oligo #10 [SEQ ID NO:24]

10 The assembled oligonucleotides create NcoI and EcoRV  
restriction ends and the DNA sequence that encodes amino  
acids 15-46 of (15-125)hIL-3 with the following amino  
acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A and 45V.  
The codons encoding amino acids 15-46 of (15-125)hIL-3  
are those found in the hIL-3 cDNA sequence except at  
15 those positions where amino acid substitutions were  
made. The 4190 base pair NcoI,EcoRV restriction fragment  
from pMON13356 was ligated with the pairs of annealed  
oligonucleotides. The ligation reaction was digested  
with NdeI and subsequently used to transform E. coli K-12  
20 strain JM101. Transformant bacteria were selected on  
ampicillin-containing plates. Plasmid DNA was isolated  
from a colony grown in LB broth. The DNA sequence was  
determined to be that of the oligonucleotides. The  
plasmid, pMON13344, encodes the (15-125)hIL-3 variant  
25 with the following amino acid sequence:

**Peptide #2**

30 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
35 Glu Asp Val Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
40 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
45

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

5 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:66]

DNA sequence #10 [SEQ ID NO:106] codes for the foregoing pMON13344 polypeptide.

EXAMPLE 9

10 Construction of pMON13345

The 4190 base pair NcoI, EcoRV restriction fragment from pMON13356 was ligated with the following pairs of annealed complementary oligonucleotides:

Oligo #1 [SEQ ID NO:15]

15 Oligo #2 [SEQ ID NO:16]

Oligo #5 [SEQ ID NO:19]

Oligo #6 [SEQ ID NO:20]

20 Oligo #11 [SEQ ID NO:25]

Oligo #12 [SEQ ID NO:26]

The assembled oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125)hIL-3 with the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S and 45M. The codons encoding amino acids 15-46 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101.

30 Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13345, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #3

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

5 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
10 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
15 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:67]

**DNA sequence #11** [SEQ ID NO:107] codes for the  
25 foregoing pMON13345 polypeptide.

#### EXAMPLE 10

##### Construction of pMON13346

The 4190 base pair NcoI, EcoRV restriction fragment from  
30 pMON13356 was ligated with the following pairs of  
annealed complementary oligonucleotides:

**Oligo #1** [SEQ ID NO:15]

**Oligo #2** [SEQ ID NO:16]

35 **Oligo #7** [SEQ ID NO:21]

**Oligo #8** [SEQ ID NO:22]

**Oligo #11** [SEQ ID NO:25]

**Oligo #12** [SEQ ID NO:26]

40

The assembled oligonucleotides create NcoI and EcoRV  
restriction ends and the DNA sequence that encodes amino  
acids 15-46 of (15-125)hIL-3 with the following amino  
acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S and 45M.

45 The codons encoding amino acids 15-46 of (15-125)hIL-3  
are those found in the hIL-3 cDNA sequence except at

those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101.

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth and DNA sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13346, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

10 **Peptide #4**

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

15 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

20 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

25 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

30 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:68]

35 **DNA sequence #12** [SEQ ID NO:108] codes for the foregoing pMON13346 polypeptide.

EXAMPLE 11

Construction of pMON13357

40 Plasmid pMON5988 DNA was digested with restriction enzymes EcoRV and NsiI, and the resulting 4218 base pair EcoRV, NsiI fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pArABAD promoter, g10L ribosome binding site, lamB secretion leader and the bases

45 encoding amino acids 15-46 and 72-125 of (15-125)hIL-3.

The 4218 base pair EcoRV, NsiI restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides:

- 5    **Oligo #19** [SEQ ID NO:33]  
     **Oligo #20** [SEQ ID NO:34]

10    The ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid  
15    the 71 bases between the EcoRV and NsiI restriction sites in the (15-125)hIL-3 gene are replaced with 22 bases from the above mentioned oligonucleotides. This linker also contains a NdeI recognition sequence.

EXAMPLE 12Construction of pMON13347

The 4218 base pair EcoRV, NsiI restriction fragment from pMON13357 was ligated with the following pairs of annealed  
 5 complementary oligonucleotides:

Oligo #21 [SEQ ID NO:35]

Oligo #22 [SEQ ID NO:36]

10 Oligo #25 [SEQ ID NO:39]

Oligo #26 [SEQ ID NO:40]

Oligo #31 [SEQ ID NO:45]

15 Oligo #32 [SEQ ID NO:46]

The assembled oligonucleotides create EcoRV and NsiI restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid  
 20 substitutions: 51R, 55L, 59L, 62V, 67N and 69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to  
 25 transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13347,  
 30 encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #5**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

35 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

40 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser



Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

5

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

10

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]

**DNA sequence #13** [SEQ ID NO:109] codes for the foregoing pMON13347 polypeptide.

15

### EXAMPLE 13

#### Construction of pMON13348

The 4218 base pair EcoRV, NsiI restriction fragment from pMON13357 was ligated with the following pairs of annealed complementary oligonucleotides:

**Oligo #21** [SEQ ID NO:35]

**Oligo #22** [SEQ ID NO:36]

25 

**Oligo #27** [SEQ ID NO:41]

**Oligo #28** [SEQ ID NO:42]

**Oligo #31** [SEQ ID NO:45]

**Oligo #32** [SEQ ID NO:46]

30

The assembled oligonucleotides create EcoRV and NsiI restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid substitutions: 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was

40

that of the oligonucleotides. The plasmid, pMON13348, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #6**

5           Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
  
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
10           Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
  
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
15           Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
  
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
20           Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
  
25           Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:70]

**DNA sequence #14** [SEQ ID NO:110] encodes the foregoing pMON13348 polypeptide.

30

**EXAMPLE 14**

**Construction of pMON13349**

The 4218 base pair EcoRV, NsiI restriction fragment from  
35           pMON13357 was ligated with the following pairs of annealed  
complementary oligonucleotides:

**Oligo #23** [SEQ ID NO:37]  
**Oligo #24** [SEQ ID NO:38]  
40             
**Oligo #25** [SEQ ID NO:39]  
**Oligo #26** [SEQ ID NO:40]  
  
**Oligo #29** [SEQ ID NO:43]  
45           **Oligo #30** [SEQ ID NO:44]

The assembled oligonucleotides create EcoRV and NsiI restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid substitutions: 51R, 55T, 59L, 62V, 67H and 69E. The

5 codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant

10 bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth and the DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13349, encodes the (15-125)hIL-3 variant with the following amino

15 acid sequence:

**Peptide #7**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

20 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn

25 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

30 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

35 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:71]

40 **DNA sequence #15** [SEQ ID NO:111] encodes the foregoing pMON13349 polypeptide.

EXAMPLE 15Construction of pMON13358

Plasmid pMON5988 DNA was digested with restriction enzymes NsiI and EcoRI and the resulting 4178 base pair NsiI,EcoRI  
5 fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB  
secretion leader and the bases encoding amino acids 15-71  
10 and 106-125 of (15-125)hIL-3. The 4178 base pair NsiI,EcoRI restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides.  
**Oligo #15** [SEQ ID NO:29]

15 **Oligo #16** [SEQ ID NO:30]

The ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were  
selected on ampicillin-containing plates. Plasmid DNA was  
20 isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 111 bases  
between the NsiI and EcoRI restriction sites in the (15-  
25 125) hIL-3 gene are replaced with 24 bases from the above mentioned oligonucleotides. This linker also contains a NdeI recognition sequence.

30 EXAMPLE 16

Construction of pMON13350

The 4178 base pair NsiI,EcoRI restriction fragment from pMON13358 was ligated with the following pairs of annealed  
complementary oligonucleotides:

35

**Oligo #41** [SEQ ID NO:55]

**Oligo #42** [SEQ ID NO:56]

Oligo #39 [SEQ ID NO:53]

Oligo #40 [SEQ ID NO:54]

5 Oligo #35 [SEQ ID NO:49]

Oligo #36 [SEQ ID NO:50]

Oligo #43 [SEQ ID NO:57]

Oligo #44 [SEQ ID NO:58]

10

The assembled oligonucleotides create NsiI and EcoRI restriction ends and the DNA sequence that encodes amino acids 72-105 of (15-125)hIL-3 with the following amino acid substitutions: 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A and 105Q. The codons encoding amino acids 72-105 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101.

15

20

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13350, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

25

**Peptide #8**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

30

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

35

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

40

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

45

Asp Trp Gln Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:72]

- 5           **DNA sequence #16** [SEQ ID NO:112] codes for the foregoing pMON13350 polypeptide.

#### EXAMPLE 17

##### 10   Construction of pMON13355

The 4178 base pair NsiI, EcoRI restriction fragment from pMON13358 was ligated with the following pairs of annealed complementary oligonucleotides:

- 15   **Oligo #41** [SEQ ID NO:55]  
      **Oligo #42** [SEQ ID NO:56]

**Oligo #37** [SEQ ID NO:51]  
      **Oligo #38** [SEQ ID NO:52]

20

**Oligo #33** [SEQ ID NO:47]  
      **Oligo #34** [SEQ ID NO:48]

**Oligo #43** [SEQ ID NO:57]

- 25   **Oligo #44** [SEQ ID NO:58]

- The assembled oligonucleotides create NsiI and EcoRI restriction ends and the DNA sequence that encodes amino acids 72-105 of (15-125)hIL-3 with the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A and 105Q. The codons encoding amino acids 72-105 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101.
- 30
- 35

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine

that the sequence was that of the oligonucleotides. The plasmid, pMON13355, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

5 **Peptide #9**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 10 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 15 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 Asp Trp Gln Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:73]

**DNA sequence #17** [SEQ ID NO:113] codes for the foregoing pMON13355 polypeptide.  
 30

EXAMPLE 18

Construction of pMON13359

Plasmid pMON5988 DNA was digested with restriction enzymes  
 35 EcoRI and HindIII, and the resulting 4225 base pair  
 EcoRI, HindIII fragment contains the following genetic  
 elements: beta-lactamase gene (AMP), pBR327 origin of  
 replication, phage f1 origin of replication as the  
 transcription terminator, pAraBAD promoter, g10L ribosome  
 40 binding site, lamB secretion leader and the bases encoding  
 amino acids 15-105 of (15-125)hIL-3. The 4225 base pair  
 EcoRI, HindIII restriction fragment from pMON5988 was  
 ligated to the following annealed complementary  
 oligonucleotides.

45

**Oligo #17** [SEQ ID NO:31]

Oligo #18 [SEQ ID NO:32]

5 The ligation reaction was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double  
10 digest. In the resulting plasmid the 64 bases between the EcoRI and HindIII restriction sites in the (15-125)hIL-3 gene are replaced with 20 bases from the above mentioned oligonucleotides. This linker also contains an NdeI recognition sequence.

15

#### EXAMPLE 19

##### Construction of pMON13352

20 The 4225 base pair EcoRI, HindIII restriction fragment from pMON13359 was ligated with the following pairs of annealed complementary oligonucleotides:

Oligo #45 [SEQ ID NO:59]

Oligo #46 [SEQ ID NO:60]

25

Oligo #49 [SEQ ID NO:63]

Oligo #50 [SEQ ID NO:64]

30 The assembled oligonucleotides create EcoRI and HindIII restriction ends and the DNA sequence that encodes amino acids 106-125 of (15-125)hIL-3 with the following amino acid substitutions: 109E, 116V, 120Q and 123E. The codons encoding amino acids 106-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions  
35 where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were



selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13352, encodes the  
 5 (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #10**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 10 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 15 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 20 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 25 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:74]

30 **DNA sequence #18** [SEQ ID NO:114] codes for the foregoing pMON13352 polypeptide.

**EXAMPLE 20**35 **Construction of pMON13354**

The 4225 base pair EcoRI, HindIII restriction fragment from pMON13359 was ligated with the following pairs of annealed complementary oligonucleotides:

40 **Oligo #45** [SEQ ID NO:59]  
**Oligo #46** [SEQ ID NO:60]

**Oligo #47** [SEQ ID NO:61]  
**Oligo #48** [SEQ ID NO:62]

45 The assembled oligonucleotides create EcoRI and HindIII

restriction ends and the DNA sequence that encodes amino acids 106-125 of (15-125)hIL-3 with the following amino acid substitutions: 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 106-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth, and the DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13354, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

15 **Peptide #11**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:75]

**DNA sequence #19** [SEQ ID NO:115] codes for the foregoing pMON13354 polypeptide.

EXAMPLE 21

Construction of pMON13360

45 Plasmid pMON13352 DNA was digested with restriction enzymes NsiI and EcoRI, resulting in a 4178 base pair

NsiI, EcoRI fragment. The genetic elements derived from pMON13352 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-71 and 106-125 of (15-125)hIL-3. Plasmid pMON13350 DNA was digested with NsiI and EcoRI. The resulting 111 base pair NsiI, EcoRI fragment encodes amino acids 72-105 of (15-125)hIL-3. The eluted restriction fragments were concentrated and desalted using Centricon 30 concentrators. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and analyzed by restriction analysis. Clones containing the correct insert lost a XmnI site as compared with pMON13352. Positive clones were identified by the loss of a 615 base pair XmnI fragment. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13360, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #12**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

5

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ. NO:76]

**DNA sequence #23** [SEQ ID NO:119] encodes the foregoing pMON13360 polypeptide.

10

## EXAMPLE 22

### Construction of pMON13361

Plasmid pMON13352 DNA was digested with restriction  
 15 enzymes NsiI and EcoRI, resulting in a 4178 base pair  
 NsiI,EcoRI fragment. The genetic elements derived from  
 pMON13352 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, pAraBAD promoter, g10L ribosome  
 20 binding site, lamB secretion leader and the bases encoding  
 amino acids 15-71 and 106-125 of (15-125)hIL-3. Plasmid  
 pMON13355 DNA was digested with NsiI and EcoRI. The  
 resulting 111 base pair NsiI, EcoRI fragment encodes amino  
 acids 72-105 of (15-125)hIL-3. The restriction fragments  
 25 were ligated, and the ligation reaction mixture was used  
 to transform E. coli K-12 strain JM101. Transformant  
 bacteria were selected on ampicillin-containing plates.  
 Clones containing the correct insert contained an  
 additional RsaI site which results in a 1200 base pairs  
 30 RsaI fragment. The DNA was sequenced to confirm the  
 correct insert. The resulting (15-125)hIL-3 variant has  
 the following amino acid substitutions: 73G, 76A, 79R,  
 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E.  
 The codons encoding amino acids 72-125 of (15-125)hIL-3  
 35 are those found in the hIL-3 cDNA sequence except at those  
 positions where amino acid substitutions were made. The  
 plasmid, pMON13361, encodes the (15-125)hIL-3 variant with  
 the following amino acid sequence:

### **Peptide #13**

40

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 5 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 10 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 20 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:77]  
 DNA sequence #24 [SEQ ID NO:120] codes for the  
 foregoing pMON13361 polypeptide.

25

EXAMPLE 23Construction of pMON13362

Plasmid pMON13354 DNA was digested with restriction  
 enzymes NsiI and EcoRI, resulting in a 4178 base pair  
 30 NsiI,EcoRI fragment. The genetic elements derived from  
 pMON13354 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage fl origin of replication as the  
 transcription terminator, pAraBAD promoter, gl0L ribosome  
 binding site, lamB secretion leader and the bases encoding  
 35 amino acids 15-71 and 106-125 of (15-125)hIL-3. Plasmid  
 pMON13355 DNA was digested with NsiI and EcoRI. The  
 resulting 111 base pair NsiI, EcoRI fragment encodes amino  
 acids 72-105 of (15-125)hIL-3. The restriction fragments  
 were ligated, and the ligation reaction mixture was used  
 40 to transform E. coli K-12 strain JM101. Transformant  
 bacteria were selected on ampicillin-containing plates.  
 Clones containing the correct insert contained an  
 additional RsaI site which results in a 1200 base pairs  
 RsaI fragment. The DNA was sequenced to confirm the  
 45 correct insert. The resulting (15-125)hIL-3 variant has

the following amino acid substitutions: 75G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13362, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #14**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:78]

**DNA sequence #25** [SEQ ID NO:121] codes for the foregoing pMON13362 polypeptide.

**EXAMPLE 24****Construction of pMON13363**

Plasmid pMON13344 DNA was digested with restriction enzymes NsiI and EcoRV, resulting in a 4218 base pair NsiI, EcoRV fragment. The genetic elements derived from pMON13344 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. Plasmid pMON13348 DNA was digested with NsiI and EcoRV. The

resulting 70 base pair NsiI, EcoRV fragment encodes amino acids 47-71 of (15-125)hIL-3. The restriction fragments were ligated with T4 ligase, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101.

5 Transformant bacteria were selected on ampicillin-containing plates. Clones containing the correct insert contained an additional DdeI site which results in DdeI restriction fragments of 806 and 167 base pairs compared to 973 base pairs in pMON13344. The DNA was sequenced to  
10 confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence  
15 except at those positions where amino acid substitutions were made. The plasmid, pMON13363, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #15**

20 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
25 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
30 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
35 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
40 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:79]

**DNA sequence #20** [SEQ ID NO:116] codes for the foregoing pMON13363 polypeptide.

45

EXAMPLE 25

Construction of pMON13364

Plasmid pMON13345 DNA was digested with restriction enzymes NsiI and EcoRV, resulting in a 4218 base pair NsiI, EcoRV fragment. The genetic elements derived from pMON13345 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. Plasmid pMON13349 DNA was digested with NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV fragment encodes amino acids 47-71 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Clones containing the correct insert contained an additional DdeI site which results in DdeI restriction fragments of 806 and 167 base pairs compared to 973 base pairs in pMON13344. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13364, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #16**

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly



Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

5

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:80]

**DNA sequence #21** [SEQ ID NO:117] codes for the foregoing pMON13364 polypeptide.

10

#### EXAMPLE 26

##### Construction of pMON13365

Plasmid pMON13346 DNA was digested with restriction  
 15 enzymes NsiI and EcoRV, resulting in a 4218 base pair  
 NsiI, EcoRV fragment. The genetic elements derived from  
 pMON13346 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, pAraBAD promoter, g10L ribosome  
 20 binding site, lamB secretion leader and the bases encoding  
 amino acids 15-46 and 72-125 of (15-125)hIL-3. Plasmid  
 pMON13347 DNA was digested with NsiI and EcoRV. The  
 resulting 71 base pair NsiI, EcoRV fragment encodes amino  
 acids 47-71 of (15-125)hIL-3. The restriction fragments  
 25 were ligated, and the ligation reaction mixture was used  
 to transform E. coli K-12 strain JM101. Transformant  
 bacteria were selected on ampicillin-containing plates.  
 Clones containing the correct insert contained an  
 additional DdeI site which results in DdeI restriction  
 30 fragments of 806 and 167 base pairs compared to 973 base  
 pairs in pMON13344. The DNA was sequenced to confirm the  
 correct insert. The resulting (15-125)hIL-3 variant has  
 the following amino acid substitutions: 18I, 25H, 29V,  
 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E. The  
 35 codons encoding amino acids 15-71 of (15-125)hIL-3 are  
 those found in the hIL-3 cDNA sequence except at those  
 positions where amino acid substitutions were made. The  
 plasmid, pMON13365, encodes the (15-125)hIL-3 variant with  
 the following amino acid sequence:

**Peptide #**

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

5 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
10 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
15 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
20 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:81]  
DNA sequence #22 [SEQ ID NO:118] codes for the  
25 foregoing pMON13365 polypeptide.

EXAMPLE 27Construction of pMON13298

Plasmid pMON5978 DNA was digested with restriction enzymes NsiI and HindIII, resulting in a 3789 base pair

5 NsiI,HindIII fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter, g10L ribosome binding site, and the bases encoding amino acids 15-71 of

10 (15-125)hIL-3. Plasmid pMON13360 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform

15 E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G,

20 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13298, encodes the (15-

25 125)hIL-3 variant with the following amino acid sequence:

**Peptide #18**

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

30 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

35 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

40 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

45 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:82]

- 5           **DNA sequence #29** [SEQ ID NO:125] codes for the foregoing pMON13298 polypeptide.

#### EXAMPLE 28

##### 10   Construction of pMON13299

Plasmid pMON5978 DNA was digested with restriction enzymes NsiI and HindIII, resulting in a 3789 base pair NsiI, HindIII fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preCA promoter, g10L ribosome binding site and the bases encoding amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA was digested with NsiI and HindIII, the resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13299, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

##### **Peptide #19**

35   Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

40   Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

5 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:83]  
 15 **DNA sequence #30** [SEQ ID NO:126] codes for the  
 foregoing pMON13299 polypeptide.

20

EXAMPLE 29Construction of pMON13300

Plasmid pMON5978 DNA was digested with restriction enzymes  
 NsiI and HindIII, resulting in a 3789 base pair  
 25 NsiI,HindIII fragment. The genetic elements derived from  
 pMON5978 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, precA promoter, g10L ribosome  
 binding site, and the bases encoding amino acids 15-71 of  
 30 (15-125)hIL-3. Plasmid pMON13362 DNA was digested with  
 NsiI and HindIII. The resulting 175 base pair NsiI,  
 HindIII fragment encodes amino acids 72-125 of (15-  
 125)hIL-3. The restriction fragments were ligated, and  
 the ligation reaction mixture was used to transform  
 35 E. coli K-12 strain JM101. Transformant bacteria were  
 selected on ampicillin-containing plates. Plasmid DNA was  
 isolated, analyzed by restriction analysis, and sequenced  
 to confirm the correct insert. The resulting (15-125)hIL-  
 3 variant has the following amino acid substitutions: 73G,  
 40 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V,  
 117S, 120H and 123E. The codons encoding amino acids 72-  
 125 of (15-125)hIL-3 are those found in the hIL-3 cDNA  
 sequence except at those positions where amino acid

substitutions were made. The plasmid, pMON13300, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #20**

5 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu  
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly  
 10 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn  
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser  
 15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 25 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:84]

**DNA sequence #31** [SEQ ID NO:127] codes for the foregoing pMON13300 polypeptide.

30

EXAMPLE 30

Construction of pMON13301

Plasmid pMON5978 DNA was digested with restriction enzymes  
 35 NcoI and NsiI, resulting in a 3794 base pair NcoI, NsiI  
 fragment. The genetic elements derived from pMON5978 are  
 the beta-lactamase gene (AMP), pBR327 origin of  
 replication, phage fl origin of replication as the  
 transcription terminator, preCA promoter, g10L ribosome  
 40 binding site and the bases encoding amino acids 72-125 of  
 (15-125)hIL-3. Plasmid pMON13363 DNA was digested with  
 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI  
 fragment encodes amino acids 15-71 of (15-125)hIL-3. The  
 restriction fragments were ligated, and the ligation  
 45 reaction mixture was used to transform E. coli K-12 strain  
 JM101. Transformant bacteria were selected on ampicillin-

containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13301, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #21**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala  
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:85]

**DNA sequence #26** [SEQ ID NO:122] codes for the foregoing pMON13301 polypeptide.

**EXAMPLE 31****Construction of pMON13302**

Plasmid pMON5978 DNA was digested with restriction enzymes NcoI and NsiI, resulting in a 3794 base pair NcoI, NsiI fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preCA promoter, g10L ribosome

binding site, and the bases encoding amino acids 72-125 of (15-125)hIL-3. Plasmid pMON13364 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13302, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #22**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr  
Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:86]

**DNA sequence #27** [SEQ ID NO:123] codes for the foregoing pMON13302 polypeptide.

**EXAMPLE 32**



Construction of pMON13303

Plasmid pMON5978 DNA was digested with restriction enzymes NcoI and NsiI, resulting in a 3794 base pair NcoI, NsiI fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preCA promoter, g10L ribosome binding site, and the bases encoding amino acids 72-125 of (15-125)hIL-3. Plasmid pMON13365 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13303, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #23**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu  
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Asn Gln Ala Gln Gln [SEQ ID NO:87]

**DNA sequence #28** [SEQ ID NO:124] codes for the foregoing pMON13303 polypeptide.

5

### EXAMPLE 33

#### Construction of pMON13287

Plasmid pMON2341 DNA was digested with restriction enzymes  
 10 NcoI and HindIII, resulting in a 3619 base pair  
 NcoI,HindIII fragment. The genetic elements derived from  
 pMON2341 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, precA promoter and gl0L ribosome  
 15 binding site. Plasmid pMON13363 DNA was digested with  
 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI  
 fragment encodes amino acids 15-71 of (15-125)hIL-3.  
 Plasmid pMON13360 DNA was digested with NsiI and HindIII.  
 The resulting 175 base pair NsiI, HindIII fragment encodes  
 20 amino acids 72-125 of (15-125)hIL-3. The restriction  
 fragments were ligated, and the ligation reaction mixture  
 was used to transform E. coli K-12 strain JM101.  
 Transformant bacteria were selected on ampicillin-  
 containing plates. Plasmid DNA was isolated, analyzed by  
 25 restriction analysis, and sequenced to confirm the correct  
 insert. The resulting (15-125)hIL-3 variant has the  
 following amino acid substitutions: 18I, 25H, 29R, 32A,  
 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A,  
 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and  
 30 123E. The codons encoding amino acids 15-125 of (15-  
 125)hIL-3 are those found in the hIL-3 cDNA sequence  
 except at those positions where amino acid substitutions  
 were made. The plasmid, pMON13287, encodes the (15-  
 125)hIL-3 variant with the following amino acid sequence:

#### 35 **Peptide #24**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:88]

20 **DNA sequence #1** [SEQ ID NO:97] codes for the  
foregoing pMON13287 polypeptide.

#### EXAMPLE 34

##### 25 Construction of pMON13288

Plasmid pMON2341 DNA was digested with restriction enzymes  
NcoI and HindIII, resulting in a 3619 base pair  
NcoI, HindIII fragment. The genetic elements derived from  
pMON2341 are the beta-lactamase gene (AMP), pBR327 origin  
30 of replication, phage f1 origin of replication as the  
transcription terminator, preC promoter and g10L ribosome  
binding site. Plasmid pMON13364 DNA was digested with  
NcoI and NsiI. The resulting 170 base pair NcoI, NsiI  
fragment encodes amino acids 15-71 of (15-125)hIL-3.  
35 Plasmid pMON13360 DNA was digested with NsiI and HindIII.  
The resulting 175 base pair NsiI, HindIII fragment encodes  
amino acids 72-125 of (15-125)hIL-3. The restriction  
fragments were ligated, and the ligation reaction mixture  
was used to transform E. coli K-12 strain JM101.  
40 Transformant bacteria were selected on ampicillin-  
containing plates. Plasmid DNA was isolated, analyzed by  
restriction analysis, and sequenced to confirm the correct  
insert. The resulting (15-125)hIL-3 variant has the  
following amino acid substitutions: 18I, 25H, 29R, 32N,

37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69S, 73G, 76A,  
 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and  
 123E. The codons encoding amino acids 15-125 of (15-  
 125)hIL-3 are those found in the hIL-3 cDNA sequence  
 5 except at those positions where amino acid substitutions  
 were made. The plasmid, pMON13288, encodes the (15-  
 125)hIL-3 variant with the following amino acid sequence:

**Peptide #25**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 10 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 15 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
 25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:89]

**DNA sequence #4** [SEQ ID NO:100] codes for the  
 foregoing pMON13288 polypeptide.

35

**EXAMPLE 35****Construction of pMON13289**

Plasmid pMON2341 DNA was digested with restriction enzymes  
 NcoI and HindIII, resulting in a 3619 base pair  
 40 NcoI,HindIII fragment. The genetic elements derived from  
 pMON2341 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, precA promoter and g10L ribosome  
 binding site. Plasmid pMON13365 DNA was digested with  
 45 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI  
 fragment encodes amino acids 15-71 of (15-125)hIL-3.

Plasmid pMON13360 DNA was digested with ~~1~~ and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101.

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13289, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #26**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser  
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly  
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:90]

**DNA sequence #7** [SEQ ID NO:103] codes for the foregoing pMON13289 polypeptide.

EXAMPLE 36Construction of pMON13290

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair  
5 NcoI, HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preC promoter and g10L ribosome binding site. Plasmid pMON13363 DNA was digested with  
10 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction  
15 fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct  
20 insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and  
25 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13290, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #27**

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala

35 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

40 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

5 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:91]

10 **DNA sequence #2** [SEQ ID NO:98] codes for the  
foregoing pMON13290 polypeptide.

EXAMPLE 37Construction of pMON13292

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair

5 NcoI, HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preC promoter and gl0L ribosome binding site. Plasmid pMON13365 DNA was digested with

10 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction

15 fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct

20 insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-

25 125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13292, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #28**

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

35 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

40 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser



Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

5 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:92]

10 **DNA sequence #8** [SEQ ID NO:104] codes for the foregoing pMON13292 polypeptide.

#### EXAMPLE 38

##### 15 Construction of pMON13294

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI, HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preCA promoter and gl0L ribosome binding site. Plasmid pMON13364 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3.

25 Plasmid pMON13362 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101.

30 Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N,

35 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions

40 were made. The plasmid, pMON13294, encodes the (15-

125)hIL-3 variant with the following amino acid sequence:

**Peptide #29**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

5 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:93]

25 **DNA sequence #6** [SEQ ID NO:102] codes for the  
 foregoing pMON13294 polypeptide.

30

**EXAMPLE 39**

**Construction of pMON13295**

Plasmid pMON2341 DNA was digested with restriction enzymes  
 NcoI and HindIII, resulting in a 3619 base pair  
 35 NcoI,HindIII fragment. The genetic elements derived from  
 pMON2341 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, preCA promoter and g10L ribosome  
 binding site. Plasmid pMON13365 DNA was digested with  
 40 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI  
 fragment encodes amino acids 15-71 of (15-125)hIL-3.  
 Plasmid pMON13362 DNA was digested with NsiI and HindIII.  
 The resulting 175 base pair NsiI, HindIII fragment encodes  
 amino acids 72-125 of (15-125)hIL-3. The restriction  
 45 fragments were ligated, and the ligation reaction mixture  
 was used to transform E. coli K-12 strain JM101.

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13295, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #30**

15 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser  
 20 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn  
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser  
 25 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser  
 35 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:94]

**DNA sequence #9** [SEQ ID NO:105] codes for the foregoing pMON13295 polypeptide.

40

#### EXAMPLE 40

##### Construction of pMON13312

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI, HindIII fragment. The genetic elements derived from

45

pMON2341 are the beta-lactamase gene (AMP<sup>r</sup> pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13364 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13312, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

**Peptide #31**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu  
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser  
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn  
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser  
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser  
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly  
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr  
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:95]

DNA sequence #5 [SEQ ID NO:101] codes for the foregoing pMON13312 polypeptide.

5

#### EXAMPLE 41

##### Construction of pMON13313

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair  
 10 NcoI, HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and gl0L ribosome binding site. Plasmid pMON13363 DNA was digested with  
 15 NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13362 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction  
 20 fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct  
 25 insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of  
 30 (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13313, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

##### **Peptide #32**

35

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

40

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser

10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser

Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:96]

**DNA sequence #3** [SEQ ID NO:99] codes for the  
foregoing pMON13313 polypeptide.

20

EXAMPLE 42Construction of pMON5987

Plasmid pMON6458 DNA was digested with restriction enzymes  
 5 NcoI and HindIII, resulting in a 3940 base pair  
 NcoI,HindIII fragment. The genetic elements derived from  
 pMON6458 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication, phage f1 origin of replication as the  
 transcription terminator, pAraBAD promoter, gl0L ribosome  
 10 binding site and lamB secretion leader. Plasmid pMON5978  
 DNA was digested with NcoI and NsiI. The resulting 170  
 base pair NcoI, NsiI fragment encodes amino acids 15-71 of  
 (15-125)hIL-3. Plasmid pMON5976 DNA was digested with  
 NsiI and HindIII. The resulting 175 base pair  
 15 NsiI,HindIII fragment encodes amino acids 72-125 of (15-  
 125)hIL-3. The restriction fragments were ligated, and  
 the ligation reaction mixture was used to transform  
E. coli K-12 strain JM101. Transformant bacteria were  
 selected on ampicillin-containing plates. Plasmid DNA was  
 20 isolated and screened for the restriction sites EcoRV and  
 NheI and DNA sequenced to confirm the correct insert.

EXAMPLE 4325 Construction of pMON5988

The plasmid DNA of pMON5987 was digested with NheI  
 and EcoRI, resulting in a 3903 base pair NheI, EcoRI  
 fragment. The 3903 base pair NheI, EcoRI fragment was  
 ligated to 1.0 picomoles of the following annealed  
 30 oligonucleotides:

5'-CTAGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA-  
 3'-GGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTATAGTT-

35 GGACGGTGACTGGAATG-3' [SEQ ID NO:131]  
 CCTGCCACTGACCTTACAATT-5' [SEQ ID NO:132]

The ligation reaction mixture was used to transform *E. coli* K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and sequenced to confirm positive clones. This  
 5 plasmid was constructed to change alanine 101 to aspartic acid in the hIL-3 gene (15-125). This plasmid was designated pMON5988.

10

EXAMPLE 44

Construction of pMON5853 (Fig 6) which encodes [Met-(15-133)hIL-3(Arg129)]

Plasmid DNA of pMON5847 (Example 2) was treated with NcoI. The restriction enzyme was inactivated by heat  
 15 treatment (65°C for 10 minutes). The DNA was then treated with large fragment of DNA polymerase I (Klenow) in the presence of all four nucleotide precursors. This produces DNA termini with non-overlapping ends. After 5 minutes at 37°C, the polymerase was inactivated by heat treatment at  
 20 65°C for 10 minutes. The DNA was then treated with HpaI, an enzyme which produces non-overlapping termini. The DNA was ethanol precipitated and ligated. The ligation reaction mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked and  
 25 plasmid DNA was analyzed by restriction analysis. A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction of the ribosome binding site to the (15-133) hIL-3 gene was  
 30 determined to be the following:

5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:133]

M N C S N [SEQ ID NO:134]

35

The lower line contains the one letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine,



asparagine, cysteine, serine and asparagine.

When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg129) which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
10 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala  
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp  
15 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys  
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg  
Leu Ala Ile Phe [SEQ ID NO:135]

20

#### EXAMPLE 45

#### Construction of pMON5873 which encodes [Met-(1-133)hIL-3]

The gene obtained from British Biotechnology, Ltd. specified arginine at codon position 129. The amino acid specified in the native hIL-3 cDNA is serine. To produce  
25 a protein with the native sequence at this position, the portion of the coding sequence between the EcoRI site at codons 106 and 107 and the NheI site at codons 129 and 130 was replaced. Plasmid DNA of pMON5854 (Example 3) and pMON5853 (Example 44) were treated with EcoRI and NheI.  
30 The larger fragments of each were gel purified. These were ligated to a pair of an annealed oligonucleotides with the following sequences:

5'-AATTCGTCGTAAGCTGACCTTCTATCTGAAAACC-  
35 3'-GGCAGCATTTGACTGGAAGATAGACTTTTGG-

TTGGAGAACGCGCAGGCTCAACAGACCACTCTGTCTG-3' [SEQ ID NO: 136]

AACCTCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATC [SEQ ID  
NO:137]

The ligation reaction mixtures were used to transform  
5 competent JM101 cells to ampicillin resistance. Colonies  
were picked into broth and grown. Plasmid DNA was  
isolated and screened for the presence of a new StyI  
recognition site present in the synthetic DNA and not in  
pMON5854 and pMON5853. The nucleotide sequence of the  
10 gene in the region between EcoRI and NheI was determined  
and found to be that of the synthetic oligonucleotides.  
The new plasmids were designated pMON5873 encoding [Met-  
(1-133)hIL-3] and pMON5872 encoding [Met-(15-133)hIL-3].

The plasmid, pMON5873, encodes Met-(1-133)hIL-3 which  
15 has the following amino acid sequence:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser  
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
20 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala  
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp  
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys  
25 Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser  
Leu Ala Ile Phe [SEQ ID NO:128]

#### EXAMPLE 46

##### 30 Construction of pMON6458

Plasmid pMON6525 was digested with restriction enzymes  
HindIII and SalI and the resulting 3172 base pair fragment  
was isolated from a 1% agarose gel by interception onto  
DEAE membrane. The genetic elements derived from pMON6525  
35 are the beta-lactamase gene (AMP), pBR327 origin of  
replication, and phage fl origin of replication as the  
transcription terminator. (The genetic elements derived

from plasmid pMON6525 are identical to those in plasmid pMON2341 which could also be used to construct pMON6458.) Plasmid pMON6457 was digested with restriction enzymes HindIII and SalI and the resulting 1117 base pair fragment was isolated by PAGE and crush and soak elution. The genetic elements derived from pMON6457 are the pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the (15-125) hIL-3 gene. The restriction fragments were ligated and the ligation reaction mixture was used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Clones containing the hIL-3 gene (encoding amino acids 15-125) contained a 345 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6458. This plasmid was constructed to eliminate an EcoRI restriction site outside the hIL-3 gene coding region in plasmid pMON6457.

#### EXAMPLE 47

##### Construction of pMON5976 which encodes [Met-(15-125)hIL-3(Ala<sup>101</sup>)]

The plasmid DNA of pMON5941 isolated from the dam-*E. coli* strain GM48 was cleaved with ClaI and NsiI and ligated to 1 picomole of an annealed assembly of six oligonucleotides encoding amino acids 20-70 of hIL-3 (FIG. 2). This synthetic fragment encodes three unique restriction sites, EcoRV, XhoI and PstI. The sequence of these oligonucleotides is shown in Figure 2.

The resulting ligation mix was used to transform competent *E. coli* JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated and the inserted fragment was determined to have both an EcoRV and NheI site. The nucleotide sequence of the region between ClaI

and NsiI was determined and found to be that of the synthetic oligonucleotides. At codons 86-87 of a nucleotide sequence coding for (15-125)hIL-3, an NheI site was introduced. The plasmid with this alteration was designated pMON5941. This plasmid encodes Met-(15-125)hIL-3 which is altered at position 101 by replacement of aspartate by alanine.

Plasmid pMON5976 encodes Met-(15-125)hIL-3(Ala101) which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala  
Ala Pro Thr Arg His Pro Ile His Ile Lys Ala Gly Asp  
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys  
Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:138]

#### EXAMPLE 48

##### Construction of pMON5917 which encodes [Met-(15-88)hIL-3]

The plasmid DNA of pMON5853 was cleaved with NsiI and HindIII and ligated to an annealed pair of oligonucleotides encoding (70-88)hIL-3 with a new NheI endonuclease restriction site at codons 86-87. The sequence of these oligonucleotides is shown in Example 18.

The ligation mixture was used to transform competent *E. coli* JM101 cells, and ampicillin resistant colonies were picked. Plasmid DNA isolated from individual colonies was screened for the presence of the new NheI restriction site. The nucleotide sequence of the substituted portion was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5917 encoding Met-(15-88)hIL-3 containing a new NheI site at codons 86-87.

Plasmid pMON5917 encodes Met-(15-88)hIL-3 which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
5 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala [SEQ ID NO:  
139]

10

EXAMPLE 49

Construction of pmon5941 which encodes [Met-(15-125)hIL-3  
Ala<sup>101</sup>]

15 The plasmid DNA of pMON5917 was cleaved with NheI and HindIII and ligated to two annealed pairs of oligonucleotides which encode amino acids 86-106 and 107-125 of hIL-3. The sequences of these oligonucleotides is shown below.

20 NheI to EcoRI

5'-CTAGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAGGCTG-  
3'-GGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTATAGTTCCGAC-

GTGACTGGAATG-3' [SEQ ID NO:140]

25 CACTGACCTTACTTAA-5' [SEQ ID NO:141]

EcoRI to HindIII

5'-AATTCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA-  
3'-GGCAGCATTGTGACTGGAAGATAGACTTTTGTGAACCTCTTGCGCGT-

30

GGCTCAACAGTAATA-3' [SEQ ID NO:142]

CCGAGTTGTCATTATTCGA-5' [SEQ ID NO:143]

35 The ligation mixture was used to transform competent *E. coli* JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be larger by

restriction analysis with NcoI and HindIII. The Asp to Ala 101 change is encoded on the NheI to EcoRI fragment. The nucleotide sequence of the portion of the coding region between the NheI and HindIII sites was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5941.

The plasmid, pMON5941, encodes Met-(15-125)hIL-3(Ala101) and contains a new NheI restriction site.

#### EXAMPLE 50

##### Construction of pMON6455

Plasmid pMON5905 was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and phage f1 origin of replication as the transcription terminator. The following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, g10L ribosome binding site and phage f1 origin of replication as the transcription terminator, derived from plasmid pMON5905 are identical to these in plasmid pMON5594 which could also be used to construct pMON6455. The AraBAD promoter is identical to that described in pMON6235. The lamB signal peptide sequence used in pMON6455 is that shown in Figure 8 fused to hIL-3 (15-125) at the NcoI site. Plasmid pMON5887 was digested with restriction enzymes HindIII and NcoI, resulting in a 384 base pair NcoI, HindIII fragment. The restriction fragments were ligated, and the ligation reaction mixture was used to transform into *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Positive clones containing the hIL-3 gene

(encoding amino acids 1-125) contained a 384 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6455.

5

EXAMPLE 51Construction of pMON6456

Plasmid pMON5905 was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site and the lamB secretion leader. Plasmid pMON5871 was digested with restriction enzymes HindIII and NcoI, resulting in a 330 base pair NcoI, HindIII fragment. The genetic element derived from pMON5871 encompassed the bases encoding the (1-107) hIL-3 gene. The restriction fragments were ligated, and the ligation reaction mixture was used to transform *E. coli* K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Clones containing the hIL-3 gene (encoding amino acids 1-107) contained a 330 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6456.

30

EXAMPLE 52Construction of pMON6457

Plasmid pMON6455 DNA grown in *E. coli* strain GM48 (dam-) was digested with restriction enzymes NcoI and ClaI, resulting in a 4263 base pair NcoI, ClaI fragment. The restriction fragment was ligated to 1.0 picomoles of annealed oligonucleotides with the following sequence

35

coding for Met Ala 14-20 hIL-3:

5'-CATGGCTAACTGCTCTAACATGAT-3' [SEQ ID NO:151]

3'-CGATTGACGAGATTGTACTAGC-5' [SEQ ID NO:152]

5

The resulting DNA was transformed into *E. coli* K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes XbaI and EcoRI in double digest. Positive clones containing the hIL-3 gene (encoding aa 15-125 of hIL-3) contained a 433 base pair XbaI, EcoRI restriction fragment. This construct was designated pMON6457. This plasmid was constructed to delete the first 14 amino acids of hIL-3. The coding sequence of the resulting gene begins as follows:

5'     ATG GCT AAC TGC...     3' [SEQ ID NO:153]  
20     Met Ala Asn Cys...     [SEQ ID NO:154]  
                            15

The first two amino acids (Methionine, Alanine) create an NcoI restriction site and a signal peptidase cleavage site between the lamB signal peptide and (15-125) hIL-3. Plasmid pMON6457 encodes (15-125) hIL-3 which has the amino acid sequence designated SEQ ID NO:65.

30

### EXAMPLE 53

#### Construction of pMON6235

One of the DNA fragments used to create this plasmid was generated by site-directed mutagenesis employing PCR techniques described previously using the following oligonucleotides, Oligo #51 [SEQ ID NO:155] and Oligo #52 [SEQ ID NO:156], were used as primers in this procedure. The template for the PCR reaction was *E. coli* strain W3110



chromosomal DNA, prepared as described Maniatis (1982). The oligonucleotide primers were designed to amplify the AraBAD promoter (Greenfield et al., 1978). The resulting DNA product was digested with the restriction enzymes  
5 SacII and BglII. The reaction mixture was purified as described previously. Plasmid, pMON5594, DNA was digested with SacII and BglII, resulting in a 4416 base pair SacII,BglII restriction fragment which contains the following genetic elements; beta-lactamase gene (AMP),  
10 pBR327 origin of replication, G10L ribosome binding site, phage f1 origin of replication as the transcription terminator and the chloramphenicol acetyl transferase (cat) gene. The 4416 base pair SacII,BglII restriction fragment from pMON5594 was ligated to the PCR-generated  
15 SacII, BglII DNA fragment. The ligation mixture was used to transform E. coli K-112 strain JM101. Positive clones contained a 323 base pair SacII,BglII fragment and were DNA sequenced to confirm that the SacII,BglII fragment was the AraBAD promoter. This construct was designated  
20 pMON6235.

#### EXAMPLE 54

##### Construction of pMON5647

25 Plasmid pMON5585 [prepared as disclosed in EP 0241446 incorporated herein by reference in its entirety] DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3273 base pair NcoI,HindIII fragment. The genetic elements derived from pMON5585 are the pBR327  
30 origin of replication, precA promoter, g10L ribosome binding protein, bovine somatotropin gene (bST), beta-lactamase gene (AMP) and T7 transcription terminator. Plasmid pMON3267 [prepared as disclosed in EP 0241446 incorporated herein by reference in its entirety] DNA was  
35 digested with NcoI and HindIII enzymes resulting in a 580 base pair NcoI,HindIII fragment which contains the porcine somatotropin (pST) gene. The restriction fragments were

ligated and the ligation reaction mixture was used to transform E. coli strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and  
 5 sequenced to confirm the correct insert.

#### EXAMPLE 55

##### Construction of pMON710

10 Plasmid pMON709 consists of a 1614 base pair AvaI, EcoRI fragment of transposon TN7, containing the streptomycin adenylyltransferase gene (Fling et al., 1985) and a pUC9 linker (XmaI, HindIII) cloned between the HindIII and EcoRI  
 15 sites of pUC19. The streptomycin adenylyltransferase gene CONfers resistance to streptomycin and spectinomycin. Plasmid pMON709 was mutagenized by oligonucleotide site-directed mutagenesis (methods described in Zoller and Smith, 1982) to introduce an EcoRV site at the 3' end of the streptomycin adenylyltransferase gene. The  
 20 oligonucleotide, **Oligo # 53** [SEQ ID NO:157], was used in this procedure to introduce the EcoRV site. The resulting plasmid was designated pMON710.

#### EXAMPLE 56

##### Construction of pMON5723

Plasmid pMON5647 DNA was digested with restriction enzymes DraI and SspI resulting in a 2916 base pair DraI, SspI fragment. The genetic elements derived from pMON5647 are  
 30 the pBR327 origin of replication, precA promoter, g10L ribosome binding protein, porcine somatotropin gene (pST) and T7 transcription terminator (Dunn and Strudier, 1983). Plasmid pMON710 DNA was digested with restriction enzymes HincII and EcoRV resulting in 940 base pair HincII, EcoRV  
 35 fragment containing the streptomycin adenylyltransferase gene which confers resistance to streptomycin and spectinomycin. The restriction fragments were ligated and

GAT to GAC and ATC to ATT respectively, destroying the EcoRV recognition site. The oligonucleotide, **Oligo # 55** [SEQ ID NO:159], was used in this procedure to eliminate the EcoRV site. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis to confirm the loss of the EcoRV site and sequenced to confirm the sequence of the (15-125) hIL-3 variant gene. The plasmid, pMON14058, encodes the (15-125) hIL-3 variant with the amino acid sequence of **PEPTIDE #25** [SEQ ID NO:89]. **DNA sequence # 33** [SEQ ID NO:161] codes for the foregoing pMON14058 polypeptide.

#### 15 EXAMPLE 59

##### Construction of pMON13438

Plasmid pMON5723 DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3278 NcoI,HindIII fragment. The genetic elements derived from pMON5723 are the pBR327 origin of replication, preC promoter, g10L ribosome binding protein, T7 transcription terminator and streptomycin adenyltransferase gene. Plasmid pMON14058 DNA was digested with NcoI and HindIII resulting in a 345 base pair NcoI,HindIII fragment which contains the (15-125) hIL-3 gene with the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 83Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The restriction fragments were ligated and the ligation reaction mixture was used to transform E. coli strain JM101. Transformant bacteria were selected on spectinomycin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and sequenced to confirm the correct insert. The plasmid, pMON13438, encodes the (15-125) hIL-3 variant with the amino acid sequence of **PEPTIDE #25** [SEQ ID NO:89]. **DNA sequence # 33** [SEQ ID NO:161] codes for the foregoing pMON13438 polypeptide.

EXAMPLE 60Construction of pMON13285

5 Plasmid pMON13252 DNA was digested with restriction enzymes NcoI and EcoRV and the resulting 3669 base pair NcoI,EcoRV fragment contains the following genetic elements; streptomycin adenyltransferase gene, pBR327  
10 origin of replication, phage f1 origin of replication as the transcription terminator, recA promoter, g10L ribosome binding site and the bases encoding amino acids 47-125 of (15-125) hIL-3 with the following amino acid substitution, 50D. The 3669 base pair NcoI,EcoRV restriction fragment  
15 from pMON13252 was ligated to the following annealed complementary oligonucleotides.

Oligo #165 [SEQ ID NO:162]

Oligo #166 [SEQ ID NO:163]

20

Oligo #167 [SEQ ID NO:164]

Oligo #168 [SEQ ID NO:165]

Oligo #169 [SEQ ID NO:166]

25 Oligo #170 [SEQ ID NO:167]

When assembled, the oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125) hIL-3 with the following amino  
30 acid substitutions; 42D, 45M and 46S. The codons encoding amino acids 15-46 of (15-125) hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13285, encodes the (15-125) hIL-3 variant with the following  
35 amino acid sequence:

Peptide #A3 [SEQ ID NO:258]

DNA sequence #A3 pMON13285 42D, 45M 46S, 50D

5 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA  
 GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT  
 CTATCCTGAT GGACAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC  
 10 CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA  
 AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC  
 15 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC  
 TTCTATCTGA AAACCTTGGG GAACGCGCAG GCTCAACAG  
 [SEQ ID NO:398]

20

EXAMPLE 61Construction of pMON13286

Plasmid pMON5978 DNA was digested with restriction enzymes  
 25 NcoI and EcoRV and the resulting 3865 base pair NcoI,EcoRV  
 fragment contains the following genetic elements; beta-  
 lactamase gene (AMP), pBR327 origin of replication, phage  
 fl origin of replication as the transcription terminator,  
 precA promoter, g10L ribosome binding site and the bases  
 30 encoding amino acids 47-125 of (15-125) hIL-3. The 3865  
 base pair NcoI,EcoRV restriction fragment from pMON5978  
 was ligated to the following annealed complementary  
 oligonucleotides.

35 Oligo #165 [SEQ ID NO:162]  
 Oligo #166 [SEQ ID NO:163]  
 Oligo #167 [SEQ ID NO:164]  
 Oligo #168 [SEQ ID NO:165]  
 40 Oligo #169 [SEQ ID NO:166]  
 Oligo #170 [SEQ ID NO:167]

When assembled, the oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125) hIL-3 with the following amino acid substitutions; 42D, 45M and 46S. The codons encoding amino acids 15-46 of (15-125) hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13286, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

10

Peptide #A4 [SEQ ID NO:259]

DNA sequence #A4 pMON13286 42D, 45M, 46S

15 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA  
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT  
CTATCCTGAT GGAAAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC  
20 CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA  
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC  
25 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC  
TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG  
[SEQ ID NO:399]

30

#### EXAMPLE 62

#### Construction of pMON13325

The 3704 base pair EcoRI, HindIII DNA fragment from plasmid pMON13286 is ligated to the 64 base pair EcoRI, HindIII DNA fragment from plasmid pMON13215. The following genetic elements are derived from pMON13286; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the transcription terminator, precA promoter, g10L ribosome binding site and the bases encoding amino acids 15-105 of the (15-125) hIL-3 gene with the following changes, 42D, 45M, and 46S.

40

The bases encoding amino acids 106-125 of the (15-125) gene with the following change, 116W, are derived from pMON13215. The resulting plasmid, pMON13325, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

Peptide # A5 [SEQ ID NO:261]

#### EXAMPLE 63

10

#### Construction of pMON13326

The 3683 base pair NcoI, EcoRI DNA fragment from plasmid pMON13215 is ligated to the 281 base pair NcoI, EcoRI DNA fragment from plasmid pMON13285. The following genetic elements are derived from pMON13215; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the transcription terminator, preCA promoter, g10L ribosome binding site and the bases encoding amino acids 106-125 of the (15-125) hIL-3 gene with the following change, 116W. The bases encoding amino acids 15-105 of the (15-125) gene with the following change, 42D, 45M, 46S and 50D derived from pMON13285. The resulting plasmid, pMON13326, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

Peptide # A6 [SEQ ID NO:262]

#### EXAMPLE 64

30

#### Construction of pMON13332

Plasmid pMON13326 DNA is digested with restriction enzymes NsiI and EcoRI and the resulting 3853 base pair NsiI, EcoRI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator,

recA promoter, gl0L ribosome binding site and the bases encoding amino acids 15-71 and 106-125 of (15-125) hIL-3 gene with the following changes 42D, 45M, 46S, 50D and 116W. The 3853 base pair NsiI, EcoRI restriction fragment from pMON13326 is ligated to the following annealed complementary oligonucleotides.

Oligo #15(A) [SEQ ID NO:168]

10 Oligo #16(A) [SEQ ID NO:169]

In the resulting plasmid the 111 bases between the NsiI and EcoRI restriction sites in the (15-125) hIL-3 gene are replaced with 24 bases from the above mentioned oligonucleotides. This linker also creates a NdeI recognition sequence.

#### EXAMPLE 65

#### 20 Construction of pMON13330

The 3846 base pair PstI, EcoRI DNA fragment from plasmid pMON13332 is ligated to the 118 base pair PstI, EcoRI DNA fragment from plasmid pMON13305. The following genetic elements are derived from pMON13332; beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, recA promoter, gl0L ribosome binding site and the bases encoding amino acids 15-69 and 106-125 of the (15-125) hIL-3 gene with the following change, 42D, 45M, 46S, 50D and 116W. The bases encoding amino acids 70-105 of the (15-125) gene with the following change, 95R, 98I and 100R are derived from pMON13305. The resulting plasmid, pMON13330, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

Peptide # A7 [SEQ ID NO:263]



EXAMPLE 66Construction of pMON13329

5

The 3846 base pair PstI, EcoRI DNA fragment from plasmid pMON13332 is ligated to the 118 base pair PstI, EcoRI DNA fragment from plasmid pMON13304. The following genetic elements are derived from pMON13332; beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, recA promoter, gl0L ribosome binding site and the bases encoding amino acids 15-69 and 106-125 of the (15-125) hIL-3 gene with the following change, 42D, 45M, 46S, and 116W. The bases encoding amino acids 70-105 of the (15-125) gene with the following change, 98I and 100R are derived from pMON13304. The resulting plasmid, pMON13329, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

20

Peptide # A8 [SEQ ID NO:406]

EXAMPLE 67

25 Construction of pMON5853 (Fig 6) which encodes [Met-(15-133)hIL-3(Arg129)]

Plasmid DNA of pMON5847 (Example 2) was treated with NcoI. The restriction enzyme was inactivated by heat treatment (65°C for 10 minutes). The DNA was then treated with large fragment of DNA polymerase I (Klenow) in the presence of all four nucleotide precursors. This produces DNA termini with non-overlapping ends. After 5 minutes at 37°C, the polymerase was inactivated by heat treatment at 65°C for 10 minutes. The DNA was then treated with HpaI, an enzyme which produces non-overlapping termini. The DNA was ethanol precipitated and ligated. The ligation

reaction mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked and plasmid DNA was analyzed by restriction analysis. A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction of the ribosome binding site to the (15-133) hIL-3 gene was determined to be the following:

10 5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:400]  
M N C S N [SEQ ID NO:401]

The lower line contains the one-letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine, asparagine, cysteine, serine and asparagine.

When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg129) which has the following amino acid sequence:

25 Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr  
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
30 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile  
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala  
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp  
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys  
Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg  
35 Leu Ala Ile Phe [SEQ ID NO:402]

## EXAMPLE 68

Construction of pMON13252

Plasmid, pMON2341, DNA was digested with restriction  
 5 enzymes NcoI and HindIII resulting in a 3619 base pair  
 NcoI/HindIII fragment. The genetic elements derived from  
 pMON2341 are the beta-lactamase gene (AMP), pBR327 origin  
 of replication F1 phage origin of replication as the  
 transcription terminator, precA, g10L ribosome binding  
 10 site. The plasmid encoding the hIL-3 (15-125) Asp<sup>(50)</sup>  
 variant, was digested with NcoI and HindIII resulting in a  
 345 base pair NcoI/HindIII fragment. This 345 Base pair  
 NcoI/HindIII fragment was ligated with the 3619 base pair  
 fragment from pMON2341 and the ligation reaction mixture  
 15 was used to transform E.coli K-12 strain JM101. Plasmid  
 DNA was isolated and screened by restriction analysis  
 using NcoI and HindIII. Positive clones contained a 345  
 base pair NcoI/HindIII fragment. This construct was  
 designated pMON13252. The plasmid, pMON13252, encodes the  
 20 (15-125)hIL-3 variant with the following amino acid  
 sequence:

PEPTIDE A10; (15-125)HIL-3 Asp<sup>(50)</sup> pMON13252

25

	Asn	Cys	Ser	Asn	Met	Ile	Asp	Glu	Ile	Ile	Thr	His	Leu
	15					20					25		
	Lys	Gln	Pro	Pro	Leu	Pro	Leu	Leu	Asp	Phe	Asn	Asn	Leu
	30					35					40		
30	Glu	Asp	Gln	Asp	Ile	Leu	Met	Asp	Asn	Asn	Leu	Arg	Arg
	45					50					55		
	Leu	Glu	Ala	Phe	Asn	Arg	Ala	Val	Lys	Ser	Leu	Gln	Asn
	60					65					70		
	Ala	Ile	Glu	Ser	Ile	Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu
35	75					80					85		
	Ala	Thr	Ala	Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys
	90					95					100		

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Leu Lys Thr  
 105 110 115  
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:407]  
 120 125

5

DNA sequence #A10 pMON13252 50D

ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA  
 GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGGT GAAGACCAAG  
 10 ATATCCTGAT GGAACAATAAC CTTTCGTCGTC CAAACCTCGA GGCATTCAAC  
 CGTGCTGTCA ACTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA  
 AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC  
 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC  
 TTCTATCTGA AAACCTTGA GAACGCGCAG GCTCAACAG  
 15 [SEQ ID NO:408]

## Examples 69-76

The variants in Table 5 were constructed by cassette  
 20 mutagenesis using methods described in the Materials and  
 Methods and the Examples contained herein, particularly  
 Examples 54-58 . Parental plasmid DNA (Table 5), digested  
 with the appropriate restriction enzymes (Table 5), was  
 ligated with the indicated annealed pairs of complementary  
 25 oligonucleotides (Table 5). The assembled oligonucleotides  
 create appropriate restriction ends and a portion of the  
 (15-125) hIL-3 gene sequence (pMON13288 [SEQ ID NO:100]).  
 Individual isolates were screened by restriction analysis  
 and DNA sequenced to confirm that the desired changes in  
 30 the (15-125) hIL-3 variant gene were made. The  
 oligonucleotides create change(s) in the (15-125) hIL-3  
 gene which encode the corresponding amino acid  
 substitution(s) in the variant polypeptide (Table 5). The  
 amino acids substitutions in addition to and/or different  
 35 from those in polypeptide # 25 [SEQ ID NO:89] are  
 indicated in Table 5. The table also shows the plasmid  
 designation (pMON number), DNA sequence identification  
 number for the mutated hIL-3 gene and the identification

number for the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 5 is shown in Table 1.

5

## Examples 77-82

The variants in Table 6 were constructed by methods described in the Materials and Methods and the Examples contained herein, particularly in Examples 60 and 61. Parental plasmid DNA (Table 6), digested with the appropriate restriction enzymes (Table 6), was ligated with the indicated restriction fragment (Table 6). Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The resulting mutated (15-125) hIL-3 genes encode the corresponding amino acid substitutions in the variant polypeptides (Table 6). The amino acids substitutions in addition to and/or different from those in polypeptide # 25 [SEQ ID NO:89] are indicated in Table 6. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 6 is shown in Table 1.

30

## Example 83

Construction of pMON13368

One of the DNA fragments to construct the plasmid, pMON13368, was generated by site-directed mutagenesis employing PCR techniques described in the Materials and Methods and the Examples contained herein, particularly Example 53. The template for the PCR reaction was plasmid,

35

pMON13289, DNA using the oligonucleotides, Oligo #B13  
18I23A25H [SEQ ID NO: 182] and Oligo #B14 2341HIN3 [SEQ ID  
NO:183], as primers. The resulting DNA product was  
digested with the restriction enzymes NcoI and HindIII.  
5 Upon completion, the digest was heated at 70°C for 15  
minutes to inactivate the enzymes. The restriction  
fragment was purified by phenol/chloroform extraction and  
precipitation with equal volume isopropanol in the  
presence of 2M NH4OAc. The oligonucleotide, Oligo #B13  
10 18I23A25H [SEQ ID NO:182], changes the codon at position  
23 of (15-125) hIL-3 variant gene pMON13289 [SEQ ID  
NO:103] from 'ATT' to 'GCA' (Ile to Ala). The 3619 base  
pair NcoI, HindIII restriction fragment from pMON2341 was  
ligated to the PCR-generated NcoI, HindIII restriction  
15 fragment. Individual isolates were screened by restriction  
analysis and DNA sequenced to confirm that the desired  
changes in the (15-125) hIL-3 variant gene were made. The  
plasmid, pMON13368, contains the (15-125) hIL-3 variant  
gene (DNA sequence #B15 [SEQ ID NO:346]) which encodes the  
20 (15-125) hIL-3 variant polypeptide with the following  
amino acid sequence:

Polypeptide #B15 [SEQ ID NO.:278]

25

#### Example 84

#### Construction of pMON13380

Plasmid, pMON13368, DNA was digested with restriction  
30 enzymes EcoRI and HindIII. The resulting 3900 base pair  
EcoRI, HindIII fragment contains the following genetic  
elements; beta-lactamase gene (AMP), pBR327 origin of  
replication, phage F1 origin of replication as the  
transcription terminator, precA promoter, g10L ribosome  
35 binding site and the DNA sequence encoding amino acids 15-  
105 of the variant pMON13368. The 3900 base pair  
EcoRI, HindIII restriction fragment from pMON13368 was

ligated to the following annealed complementary oligonucleotides.

Oligo # B48      9E12Q6V1      [SEQ ID NO:217]  
 5 Oligo # B49      9E12Q6V3      [SEQ ID NO:218]

Oligo #49      120Q123E2      [SEQ ID NO:63]  
 Oligo #50      120Q123E4      [SEQ ID NO:64]

10 When assembled, the oligonucleotides create EcoRI and  
 HindIII restriction ends and the DNA sequence that encodes  
 amino acids 106-125 of (15-125) hIL-3 with the following  
 amino acid substitution; 109E, 112Q, 116V, 120Q and 123E.  
 The codons used in the (15-125) hIL-3 gene are those found  
 15 in the hIL-3 cDNA sequence except at those positions where  
 amino acid substitutions were made. Individual isolates  
 were screened by restriction analysis and DNA sequenced to  
 confirm that the desired changes in the (15-125) hIL-3  
 variant gene were made. The plasmid, pMON13380, contains  
 20 the (15-125) hIL-3 variant gene (DNA sequence #B16 [SEQ ID  
 NO:347]) which encodes the (15-125) hIL-3 variant  
 polypeptide with the following amino acid sequence:

Polypeptide #B16 [SEQ ID NO.:279]

25

#### Example 85

#### Construction of pMON13476

30 One of the DNA fragments to construct the plasmid,  
 pMON13476, was generated by site-directed mutagenesis  
 employing PCR techniques described in the Materials and  
 Methods and the Examples contained herein, particularly  
 Example 54. The template for the PCR reaction was plasmid,  
 35 pMON13287, DNA using the oligonucleotides, Oligo #B13  
 18I23A25H [SEQ ID NO:182] and Oligo #B14 2341HIN3 [SEQ ID  
 NO.:183] as primers. The resulting DNA product was

digested with the restriction enzymes NcoI and HindIII. Upon completion, the digest was heated at 70°C for 15 minutes to inactivate the enzymes. The restriction fragment was purified by phenol/chloroform extraction and precipitation with equal volume isopropanol in the presence of 2M NH<sub>4</sub>OAc. The oligonucleotide, Oligo #B13 18I23A25H [SEQ ID NO.:182], changes the codon at position 23 of (15-125) hIL-3 variant gene, pMON13287, [SEQ ID NO:97] from 'ATT' to 'GCA' (Ile to Ala). The 3619 base pair NcoI, HindIII restriction fragment from pMON2341 was ligated to the PCR-generated NcoI, HindIII restriction fragment. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The resulting clone also contained a change, that was not designed in the mutagenic oligonucleotide, which changed the codon at position -1 from 'GCT' to 'GAT' which changes the amino acid from Alanine to Aspartic Acid. The plasmid, pMON13476, contains the (15-125) hIL-3 variant gene (DNA sequence #B52 [SEQ ID NO:303]) which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B52 [SEQ ID NO.:314]

#### Examples 86-92

The variants in Table 7 were constructed by PCR techniques using methods described in the Materials and Methods and the Example contained herein, particularly Example 51. Two sequential PCR reactions were used to create the variants. In the first PCR reaction pMON13287 plasmid DNA served as the template and the two oligonucleotides indicated in Table 7 served as the primers. Following the PCR extension reaction, the PCR product was partially purified to remove primer that was not extended. In the second PCR reaction pMON13287 plasmid



DNA served as the template, the purified product from the first PCR reaction served as one of the primers and the Oligo #B14 2341Hin3 [SEQ ID NO:183] as the second primer. The product from the second PCR reaction was partially purified and digested with restriction enzymes NcoI and HindIII and ligated with the 3619 base pair NcoI, HindIII fragment from pMON2341. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The amino acids substitutions in addition to and/or different from those in polypeptide # 24 [SEQ ID NO:88] are indicated in Table 7. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 7 is shown in Table 1.

#### Examples 93-120

The variants in Table 8 were constructed by cassette mutagenesis using methods described in the Materials and Methods and the Examples contained here, particularly Examples 54-58. Parental plasmid DNA (Table 8), digested with the appropriate restriction enzymes (Table 8), was ligated with the indicated annealed pairs of complementary oligonucleotides (Table 8). The assembled oligonucleotides create the appropriate restriction ends and a portion of (15-125) hIL-3 gene (pMON13288 [SEQ ID NO:100]) sequence. The oligonucleotides create change(s) in the (15-125) hIL-3 variant gene which encode the corresponding amino acid substitution(s); and/or deletions from the C-terminus of the variant polypeptide (Table 8). Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The amino acids substitutions in addition to and/or different from those in polypeptide #

25 [SEQ ID NO:88] are indicated in Table 5. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant

5 polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 5 is shown in Table 1.

#### Example 121

10

##### Construction of pMON13446

Plasmid, pMON13287, DNA (purified from the E. coli strain GM48 {dam-}) was digested with restriction enzymes NcoI and ClaI. The resulting 3942 base pair NcoI,ClaI fragment

15 contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the transcription terminator, preCA promoter, g10L ribosome binding site and the DNA sequence encoding amino acids 21-125 of the (15-125) hIL-3 variant

20 pMON13287. The 3942 base pair NcoI,ClaI restriction fragment from pMON13368 was ligated to the following annealed complementary oligonucleotides.

25

Oligo #B57	338UP	[SEQ ID NO:226]
Oligo #B56	338DOWN	[SEQ ID NO:225]

When assembled, the oligonucleotides create NcoI and ClaI restriction ends and the DNA sequence that encodes the

30 following 14 amino acid sequence; Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys [SEQ ID NO:403] and the DNA sequence which encodes amino acids 15-20 of the (15-125) hIL-3 variant gene, pMON13287 [SEQ ID NO:97]. The resulting variant polypeptide has a 14 amino acid N-

35 terminal extension fused to the (15-125) hIL-3 variant polypeptide, pMON13288 [SEQ ID NO: 88]. The plasmid, pMON13446, contains the (15-125) hIL-3 variant gene (DNA sequence #B53 [SEQ ID NO:404]) which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid

sequence:

Polypeptide #B53 [SEQ ID NO.:315]

5

#### Example B54

#### Construction of pMON13390

Plasmid, pMON13288, DNA (purified from the E. coli strain GM48 {dam-}) was digested with restriction enzymes NcoI and ClaI. The resulting 3942 base pair NcoI, ClaI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the transcription terminator, preCA promoter, g10L ribosome binding site and the DNA sequence encoding amino acids 21-125 of the (15-125) hIL-3 variant pMON13288. The 3942 base pair NcoI, ClaI restriction fragment from pMON13288 was ligated to the following annealed complementary oligonucleotides.

20 Oligo #B57            338UP                    [SEQ ID NO:226]

Oligo #B56            338DOWN                   [SEQ ID NO:225]

When assembled, the oligonucleotides create NcoI and ClaI restriction ends and the DNA sequence which encodes the following 14 amino acid sequence; Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys [SEQ ID NO:403] and the DNA sequence which encodes amino acids 15-20 of the (15-125) hIL-3 variant gene pMON13288 [SEQ ID NO:100]. The resulting variant has a 14 amino acid N-terminal extension fused to the (15-125) hIL-3 variant polypeptide, pMON13288 [SEQ ID NO:88]. The plasmid, pMON13390, contains the (15-125) hIL-3 variant gene (DNA sequence #B54 [SEQ ID NO.:405] which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B54 [SEQ ID NO:316]

## Examples 133-136

The variants in Table 10 were constructed by methods described in Materials and Methods and in Examples 5 contained herein, particularly Examples 54-58. Parental plasmid DNA (Table 10), digested with the appropriate restriction enzymes (Table 10) was ligated with the indicated restriction fragment containing the changes listed (Table 10). The resulting mutated (15-125) IL-3 10 genes encode the corresponding amino acid substitutions in the variant polypeptides (Table 10). The amino acid substitutions in addition to and/or different from those in polypeptide #25 [SEQ ID NO: 89] are indicated in Table 10. The biological activity (growth promoting activity in 15 AML 193 cells) for some of the variants in Table 10 is shown in Table 1.

## Examples 123-132

20 The variants in Table 9 were constructed by casset mutagenesis using methods described in Materials and Methods and in Examples 54-58 contained herein. Parental plasmid DNA (Table 9), digested with the appropriate restriction enzymes (Table 9), was ligated with the 25 indicated annealed pairs of complementary oligonucleotides (Table 9). The assembled oligonucleotides create the appropriate restriction fragment which was inserted into the (15-125) hIL-3 gene (pMON13288 [SEQ ID NO:100] between these restriction sites. The deletions or substitutions 30 encoded by the oligonucleotide in the (15-125) IL-3 gene correspond to the amino acid deletions or substitutions in the variant polypeptide (Table 9). The amino acid substitutions or deletions, in addition to and/or different from those in the polypeptide #25 [SEQ ID NO:89] 35 are indicated in Table 9. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 9 is shown in Table 1.

[illegible]

Table 5

Example	pMON number	Parental plasmid/ restriction digest	oligo pair 1,4	oligo pair 2,5	oligo pair 3,6	amino acid changes	resulting polypeptide
Example 69	pMON13406 SEQ ID NO:332	pMON13288/ NcoI, EcoRV	19Ala1 OLIGO# B1 SEQ ID NO:170 19Ala4 OLIGO# B2 SEQ ID NO:171	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45M3 OLIGO# 11 SEQ ID NO:25 42S45M6 OLIGO# 12 SEQ ID NO:26	19Ala	polypeptide B1 SEQ ID NO:264
Example 70	pMON13414 SEQ ID NO:333	pMON13288/ NcoI, EcoRV	19Ile1 OLIGO# B3 SEQ ID NO:172 19Ile4 OLIGO# B4 SEQ ID NO:173	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45M3 OLIGO# 11 SEQ ID NO:25 42S45M6 OLIGO# 12 SEQ ID NO:26	19Ile	polypeptide B2 SEQ ID NO:265
Example 71	pMON13407 SEQ ID NO:334	pMON13288/ NcoI, EcoRV	18I25H1 OLIGO# 1 SEQ ID NO:15 18I25H4 OLIGO# 2 SEQ ID NO:16	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45V3 OLIGO#B11 SEQ ID NO:180 42S45V6 OLIGO#B12 SEQ ID NO:181	45Val	polypeptide B3 SEQ ID NO:266
Example 72	pMON13405 SEQ ID NO:335	pMON13288/ NcoI, EcoRV	19Ala1 OLIGO# B1 SEQ ID NO:170 19Ala4 OLIGO# B2 SEQ ID NO:171	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45V3 OLIGO#B11 SEQ ID NO:180 42S45V6 OLIGO#B12 SEQ ID NO:181	19Ala,45Val	polypeptide B4 SEQ ID NO:267
Example 73	pMON13415 SEQ ID NO:336	pMON13288/ NcoI, EcoRV	19Ile1 OLIGO# B3 SEQ ID NO:172 19Ile4 OLIGO# B4 SEQ ID NO:173	29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20	42S45V3 OLIGO#B11 SEQ ID NO:180 42S45V6 OLIGO#B12 SEQ ID NO:181	19Ile,45Val	polypeptide B5 SEQ ID NO:268
Example 74	pMON13408 SEQ ID NO:337	pMON13288/ EcoRV, NsiI	49Ile1 OLIGO# B7 SEQ ID NO:176 49Ile4 OLIGO# B8 SEQ ID NO:177	59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40	67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44	49Ile	polypeptide B6 SEQ ID NO:269

Table 5 cont

Example 75	pMON13409 SEQ ID NO:338	pMON13288/ EcoRV, NsiI	49Leu1 SEQ ID NO:178 OLIGO# B9 49Leu4 OLIGO# B10 SEQ ID NO:179	59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40	67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44	49Leu	polypeptide B7 SEQ ID NO:270
Example 76	pMON13410 SEQ ID NO:339	pMON13288/ EcoRV, NsiI	49Asp1 OLIGO# B5 SEQ ID NO:174 49Asp4 OLIGO# B6 SEQ ID NO:175	59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40	67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44	49Asp	polypeptide B8 SEQ ID NO:271

Table 6

Example No	plasmid pMON number	Parental plasmid/ restriction digest	restriction fragment	amino acid substitutions	resulting polypeptide
Example 77	pMON13422 SEQ ID NO:340	pMON13408/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13405	19Ala, 45Val, 49Ile	polypeptide B9 SEQ ID NO:272
Example 78	pMON13423 SEQ ID NO:341	pMON13408/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13415	19Ile, 45Val, 49Ile	polypeptide B10 SEQ ID NO:273
Example 79	pMON13424 SEQ ID NO:342	pMON13409/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13405	19Ala, 45Val, 49Leu	polypeptide B11 SEQ ID NO:274
Example 80	pMON13425 SEQ ID NO:343	pMON13409/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13415	19Ile, 45Val, 49Leu	polypeptide B12 SEQ ID NO:275
Example 81	pMON13426 SEQ ID NO:344	pMON13410/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13405	19Ala, 45Val, 49Asp	polypeptide B13 SEQ ID NO:276
Example 82	pMON13429 SEQ ID NO:345	pMON13410/ NcoI, EcoRV	99 base pair NcoI, EcoRV fragment from pMON13415	19Ile, 45Val, 49Asp	polypeptide B14 SEQ ID NO:277



Table 7

Example	pMON number	template	Step one PCR primer1	Step one PCR primer2	Step two PCR primer1	Step two PCR primer2	Amino Acid Substitutions	Polypeptide
Example 86	pMON13475 SEQ ID NO:348	pMON13287	18I23A25H OLIGO# B13 SEQ ID NO:182	42D45V46S50D OLIGO# B19 SEQ ID NO:188	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	42D,46S,50D	Polypeptide # B17 SEQ ID NO 280
Example 87	pMON13366 SEQ ID NO:349	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42D45V46S50D OLIGO# B19 SEQ ID NO:188	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	42N,46S,50D	Polypeptide # B18 SEQ ID NO 281
Example 88	pMON13367 SEQ ID NO:350	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42A45V46S50D OLIGO# B17 SEQ ID NO:186	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	46S,50D	Polypeptide # B19 SEQ ID NO 282
Example 89	pMON13369 SEQ ID NO:351	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42D45V46S50D OLIGO# B21 SEQ ID NO:190	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	42D,46S,50D	Polypeptide # B20 SEQ ID NO 283
Example 90	pMON13370 SEQ ID NO:352	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42A45M46S50D OLIGO# B16 SEQ ID NO:185	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	45M,46S,50D	Polypeptide # B21 SEQ ID NO 284
Example 91	pMON13373 SEQ ID NO:353	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42D45M46S50D OLIGO# B18 SEQ ID NO:187	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	42D,45M,46S 50D	Polypeptide # B22 SEQ ID NO 285
Example 92	pMON13374 SEQ ID NO:354	pMON13287	2341NCO OLIGO# B15 SEQ ID NO:184	42S45M46S50D OLIGO# B20 SEQ ID NO:189	product from step one	2341HIN3 OLIGO# B14 SEQ ID NO:183	42S,45M46S 50D	Polypeptide # B23 SEQ ID NO 286

Table 8

Example	plasmid	parental plasmid	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	oligo pair	resulting amino acid sub(s).	polypeptide
Example 93	pMON13375 SEQ ID NO:355	pMON13287/ EcoRI, HindIII	S09E16V1 OLIGO# B50 SEQ ID NO:219 S09E16V3 OLIGO# B51 SEQ ID NO:220 S09E206V1 OLIGO# B54 SEQ ID NO:223 S09E206V3 OLIGO# B55 SEQ ID NO:224	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	15-119	polypeptide B24 SEQ ID NO:287
Example 94	pMON13376 SEQ ID NO:356	pMON13476/ EcoRI, HindIII	S09E206V1 OLIGO# B54 SEQ ID NO:223 S09E206V3 OLIGO# B55 SEQ ID NO:224	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	15-119, 23A, 112Q	polypeptide B25 SEQ ID NO:288
Example 95	pMON13377 SEQ ID NO:357	pMON13475/ EcoRI, HindIII	S09E206V1 OLIGO# B54 SEQ ID NO:223 S09E206V3 OLIGO# B55 SEQ ID NO:224	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	15-119, 23A, 42D, 46S, 50D, 112Q	polypeptide B26 SEQ ID NO:289
Example 96	pMON13378 SEQ ID NO:358	pMON13365/ EcoRI, HindIII	S09E16V1 OLIGO# B50 SEQ ID NO:219 S09E16V3 OLIGO# B51 SEQ ID NO:220	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	S116VD31 OLIGO# B52 SEQ ID NO:221 SECRID33 OLIGO# B53 SEQ ID NO:222	15-119, 23A	polypeptide B27 SEQ ID NO:290
Example 97	pMON13379 SEQ ID NO:359	pMON13367/ EcoRI, HindIII	9E1206V1 OLIGO# B48 SEQ ID NO:217 9E1206V3 OLIGO# B49 SEQ ID NO:218	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64	46S, 50D, 112Q	polypeptide B28 SEQ ID NO:291
Example 98	pMON13385 SEQ ID NO:360	pMON13287/ NcoI, EcoRV	18125H1 OLIGO# 1 SEQ ID NO:15 18125H4 OLIGO# 2 SEQ ID NO:16	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	29V, 32R, 34S	polypeptide B29 SEQ ID NO:292
Example 99	pMON13381 SEQ ID NO:361	pMON13287/ NsiI, EcoRI	73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	82W	polypeptide B30 SEQ ID NO:293

Table 8 cont

Example 100	pMON13383 SEQ ID NO:362	pMON13475/ EcoRI, HindIII	9E12Q6V1 OLIGO# B48 SEQ ID NO:217 9E12Q6V5 OLIGO# B49 SEQ ID NO:218	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64			23A, 42D, 46S, 50D 112Q	polypeptide B31 SEQ ID NO:294
Example 101	pMON13384 SEQ ID NO:363	pMON13287/ EcoRI, HindIII	9E12Q6V1 OLIGO# B48 SEQ ID NO:217 9E12Q6V5 OLIGO# B49 SEQ ID NO:218	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64			112Q	polypeptide B32 SEQ ID NO:295
Example 102	pMON13388 SEQ ID NO:364	pMON13287/ EcoRV, NsiI	50D56S1 OLIGO# B42 SEQ ID NO:211 50ASP4 OLIGO# B41 SEQ ID NO:210	60S62V2 OLIGO# 27 SEQ ID NO:41 56SER5 OLIGO# B43 SEQ ID NO:212	67N69E3 OLIGO# 31 SEQ ID NO:45 67N69E6 OLIGO# 32 SEQ ID NO:46		50D, 56S	polypeptide B33 SEQ ID NO:296
Example 103	pMON13389 SEQ ID NO:365	pMON13287/ NcoI, EcoRV	18I25H1 OLIGO#1 SEQ ID NO:15 18I25H4 OLIGO#2 SEQ ID NO:16	29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	42D45H3 OLIGO# B32 SEQ ID NO:201 42D45M6 OLIGO# B33 SEQ ID NO:202		42D, 45M	polypeptide B34 SEQ ID NO:297
Example 104	pMON13391 SEQ ID NO:366	pMON13287/ NcoI, EcoRV	18I25H1 OLIGO#1 SEQ ID NO:15 18I25H4 OLIGO#2 SEQ ID NO:16	34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200	42A45V3 OLIGO# 9 SEQ ID NO:23 42A45V6 OLIGO# 10 SEQ ID NO:24		34S	polypeptide B35 SEQ ID NO:298
Example 105	pMON13392 SEQ ID NO:367	pMON13287/ NcoI, EcoRV	18I25H1 OLIGO#1 SEQ ID NO:15 18I25H4 OLIGO#2 SEQ ID NO:16	29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	42D45V3 OLIGO# B34 SEQ ID NO:203 42D45V6 OLIGO# B35 SEQ ID NO:204		42D	polypeptide B36 SEQ ID NO:299
Example 106	pMON13393 SEQ ID NO:368	pMON13287/ NcoI, EcoRV	23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196	34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200	42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206		23A, 34S, 42D, 45M 46S	polypeptide B37 SEQ ID NO:300

Table 8 cont

Example 107	pMON13394 SEQ ID NO:369	pMON13287/ NcoI, EcoRV	18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16	29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206	42D, 45M, 46S	polypeptide B38 SEQ ID NO:301
Example 108	pMON13395 SEQ ID NO:370	pMON13287/ NcoI, EcoRV	23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208	23A, 29V, 32R, 34S 42D, 46S	polypeptide B39 SEQ ID NO:302
Example 109	pMON13396 SEQ ID NO:371	pMON13287/ NsiI, EcoRI	73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56	79R82Q2 OLIGO# 39 SEQ ID NO:53 79R82Q5 OLIGO# 40 SEQ ID NO:54	100ARG3 SEQ ID NO: 87S93S98I7 OLIGO# 36 SEQ ID NO:50	100R, 101M	polypeptide B40 SEQ ID NO:303
Example 110	pMON13397 SEQ ID NO:372	pMON13287/ NsiI, EcoRI	73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56	82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214	100ARG3 OLIGO# B22 SEQ ID NO:191 87S93S98I7 OLIGO# 36 SEQ ID NO:50	82W, 100R, 101M	polypeptide B41 SEQ ID NO:304
Example 111	pMON13398 SEQ ID NO:373	pMON13287/ NcoI, EcoRV	18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16	29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208	42D, 46S	polypeptide B42 SEQ ID NO:305
Example 112	pMON13399 SEQ ID NO:374	pMON13388/ NcoI, EcoRV	23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196	29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198	42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208	23A, 29V, 32R, 34S 42D, 46S	polypeptide B43 SEQ ID NO:306
Example 113	pMON13404 SEQ ID NO:375	pMON13287/ EcoRI, HindIII	S9E2Q6V1 OLIGO# B54 SEQ ID NO:223 S9E2Q6V3 OLIGO# B55 SEQ ID NO:224	S116VD31 OLIGO# B52 SEQ ID NO:221 S9E2Q6V3 OLIGO# 53 SEQ ID NO:222		15-119 112Q	polypeptide B44 SEQ ID NO:307

Table 8 cont

Example 114	pMON13387 SEQ ID NO:376	pMON13287/ EcoRV, NsiI	SOASP1 OLIGO# B40 SEQ ID NO:209 SOASP4 OLIGO# B41 SEQ ID NO:210 18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16	60S62V2 OLIGO# 27 SEQ ID NO:41 60S62V5 OLIGO# 28 SEQ ID NO:42 29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	67N69E3 OLIGO# 31 SEQ ID NO:45 67N69E6 OLIGO# 32 SEQ ID NO:46 42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208 42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206	50D	polypeptide B45 SEQ ID NO:308
Example 115	pMON13416 SEQ ID NO:377	pMON13387/ NcoI/EcoRV	18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16	29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208	42D, 46S, 50D	polypeptide B46 SEQ ID NO:309
Example 116	pMON13417 SEQ ID NO:378	pMON13387/ NcoI/EcoRV	18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16	29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18	42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206	42D, 45M, 46S, 50D	polypeptide B47 SEQ ID NO:310
Example 117	pMON13420 SEQ ID NO:379	pMON13388/ NcoI, EcoRV	23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196	34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200	42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208	23A, 34S, 42D, 46S, 50D, 56S	polypeptide B48 SEQ ID NO:311
Example 118	pMON13421 SEQ ID NO:380	pMON13388/ NcoI, EcoRV	23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196	34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200	42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206	23A, 34S, 42D, 45M 46S, 50D, 56S	polypeptide B49 SEQ ID NO:311
Example 119	pMON13432 SEQ ID NO:381	pMON13387/ NcoI, EcoRV	23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196	34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200	42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206	23A, 34S, 42D, 45M 46S, 50D	polypeptide B50 SEQ ID NO:312
Example 120	pMON13382 SEQ ID NO:382	pMON13287/ EcoRI, HindIII	9E1206W1 OLIGO# B46 SEQ ID NO:215 9E12016W3 OLIGO# B47 SEQ ID NO:216	120Q123E2 OLIGO# 49 SEQ ID NO:63 120Q123E4 OLIGO# 50 SEQ ID NO:64		112D, 116W	polypeptide B51 SEQ ID NO:313

TABLE 9

Example No.	Plasmid	Parental Plasmid/ Restriction Digest	Oligo pair	Oligo pair	Oligo pair	Oligo pair	Amino acid changes	Polypeptide
Example 124	pMON13400 SEQ ID NO:384	pMON13288 Restriction NcoI-EcoRV	20P23A1 SEQ ID NO:232 20P23A4 SEQ ID NO:233	29I4S7S2 SEQ ID NO:236 29I4S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	20P 23A 29I 34S 37S 38A 45V 46S	Polypeptide C-2 SEQ ID NO:317	
Example 125	pMON13402 SEQ ID NO:385	pMON13288 Restriction NcoI-EcoRV	23L1 SEQ ID NO:234 23L4 SEQ ID NO:235	29I4S7S2 SEQ ID NO:236 29I4S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	23L 29I 34S 37S 38A 45V 46S	Polypeptide C-3 SEQ ID NO:318	
Example 131	pMON13440 SEQ ID NO:386	pMON13288 Restriction NcoI-EcoRV	18I3A5H1 SEQ ID NO:195 18I3A5H4 SEQ ID NO:196	29I4S7S2 SEQ ID NO:236 29I4S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	18I 23A 26H 29I 34S 37S 38A 45V 46S	Polypeptide C-10 SEQ ID NO:319	
Example 132	pMON13451 SEQ ID NO:387	pMON13288 Restriction NcoI-EcoRV	19I0L3A1 SEQ ID NO:230 19I0L3A4 SEQ ID NO:231	29I4S7S2 SEQ ID NO:236 29I4S7S5 SEQ ID NO:237	38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239	19I 20L 23A 29I 34S 37S 38A 45V 46S	Polypeptide C-11 SEQ ID NO:320	
Example 130	pMON13419 SEQ ID NO:389	pMON13288 Restriction EcoRV-NsiI	50D5IS1 SEQ ID NO:240 50D5IS4 SEQ ID NO:241	62P3H5S2 SEQ ID NO:244 62P3H5 SEQ ID NO:246	67Q3 SEQ ID NO:248 65S67Q6 SEQ ID NO:247	50D 5IS 62P 63H 65S 67Q	Polypeptide C-8 SEQ ID NO:325	
Example 126	pMON13403 SEQ ID NO:388	pMON13288 Restriction EcoRV-NsiI	50D5IS1 SEQ ID NO:240 50D5IS4 SEQ ID NO:241	62P3H2 SEQ ID NO:245 62P3H5 SEQ ID NO:246	67Q3 SEQ ID NO:248 67Q6 SEQ ID NO:249	50D 5IS 62P 63H 67Q	Polypeptide C-4 SEQ ID NO:321	

TABLE 9

Example 123	pMON13418 SEQ ID NO:393	pMON13288 Restriction NsiI-EcoRI	76P1 SEQ ID NO:250 76P5 SEQ ID NO:251	78S2 SEQ ID NO:252 78S6 SEQ ID NO:253	5VYWPTT3 SEQ ID NO:242 5VYWPTT7 SEQ ID NO:243	101A105Q4 SEQ ID NO:57 101A105Q8 SEQ ID NO:58	76P 78S 85V 87Y 88W 91P 95T 98T	Polypeptide C-1 SEQ ID NO:326
Example 127	pMON13411 SEQ ID NO:390	pMON13288 Restriction EcoRI-HindIII	09L2Q6S1 Seq ID NO:227 09L2Q6S3 SEQ ID NO:228	120Q123E2 SEQ ID NO:63 120Q123E4 SEQ ID NO:64			109L 112Q 116S	Polypeptide C-5 SEQ ID NO:322
Example 128	pMON13412 SEQ ID NO:391	pMON13288 Restriction EcoRI-HindIII	9LQ51181 Seq ID NO:255 9LQ51183 SEQ ID NO:256				15-118 109L 112Q 116S	Polypeptide C-6 SEQ ID NO:323
Example 129	pMON13413 SEQ ID NO:392	pMON13288 Restriction EcoRI-HindIII	09L2Q6S1 Seq ID NO:227 09L2Q6S3 SEQ ID NO:228	117S2 SEQ ID NO:229 120Q123E4 SEQ ID NO:64			109L 112Q 116S 117S	Polypeptide C-7 SEQ ID NO:324

TABLE 10

Example No	Plasmid	Parental plasmid/ Restriction digest	Restriction fragment	Amino Acid changes	Polypeptide
Example 133	pMON13428 SEQ ID NO:394	pMON13411 NsiI-EcoRI	102 bp NsiI-EcoRI fragment from pMON13418	76P 79S 85V 87Y 91P 95T 98T 109L 112Q 116S	Polypeptide C-9 SEQ ID NO:327
Example 134	pMON13459 SEQ ID NO:395	pMON13428 NcoI-NsiI	170 bp NcoI-NsiI fragment from pMON13402	23L 29I 34S 37S 38A 45V 46S 76P 79S 85V 87Y 91P 95T 98T 109L 112Q 116S	Polypeptide C-12 SEQ ID NO:328
Example 135	pMON13467 SEQ ID NO:396	pMON13413 NcoI-NsiI	170 bp NcoI-NsiI fragment from pMON13402	23L 29I 34S 37S 38A 45V 46S 109L 112Q 116S 109L 112Q 116S 117S	Polypeptide C-13 SEQ ID NO:329
Example 136	pMON13492 SEQ ID NO:397	pMON13418 NcoI-NsiI	170 bp NcoI-NsiI fragment from pMON13402	23L 29I 34S 37S 38A 45V 46S 76P 79S 85V 87Y 91P 95T 98T	Polypeptide C-14 SEQ ID NO:330